3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 1,4-dioxane. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is

considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of 1,4-dioxane are indicated in Table 3-2 and Figure 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for 1,4-dioxane. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix C). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

3.2.1.1 Death

Several cases of death in humans have been documented after exposure to high concentrations of 1,4-dioxane. Barber (1934) described five deaths that occurred within a period of 2 weeks among factory workers engaged in a process that involved primarily exposure to 1,4-dioxane vapors, although minimal dermal exposure could have not been avoided. Three of the subjects suffered from abdominal pain and vomiting before death occurred. Post-mortem examination of the subjects showed extensive gross and microscopic lesions to the liver and kidneys. Based on his observations, Barber (1934) suggested that the effects on the kidneys may have been responsible for the fatal outcome and that liver necrosis, although widespread, was compatible with recovery. No exposure levels were available in these case reports. Johnstone (1959) described an additional fatal case of a worker exposed to 1,4-dioxane for only 1 week and whose post-mortem examination showed kidney and liver alterations similar to those described by Barber (1934). In the Johnstone case, the room in which the patient had worked had no exhaust ventilation and the worker was not provided a respirator. The minimum concentration of 1,4-dioxane in the room was 208 ppm and the maximum was in excess of 650 ppm; the average concentration was 470 ppm. In addition, dermal exposure may have been considerable in this case.

Studies in animals, mostly early studies, provide information on lethality of relatively high concentrations of 1,4-dioxane in several species and also indicate that the kidneys and liver, and in some cases, the lungs, are the main targets of high airborne concentrations of 1,4-dioxane. Short-term exposure to 5,000 ppm 1,4-dioxane was lethal to rats, mice, and rabbits, whereas 10,000 ppm was lethal to guinea pigs (Fairley et al. 1934). A 4-hour LC_{50} of 14,261 ppm was calculated for rats (Pozzani et al. 1959). An additional study in guinea pigs reported that the minimum period of exposure that caused the death of the majority of a group of six animals was 180 minutes to 30,000 ppm; no deaths occurred in groups exposed to up to 10,000 ppm for up to 480 minutes (Yant et al. 1930). One out of four rabbits exposed to 2,000 ppm 1,4-dioxane 3 hours/day, 5 days/week died on week 4 of exposure, and the cause of death was attributed to renal and hepatic lesions (Fairley et al. 1934).

The LOAEL values for death in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal effects in humans or animals following inhalation exposure to 1,4-dioxane. No studies were located regarding endocrine, dermal, or body weight effects in humans after inhalation exposure to 1,4-dioxane.

The highest NOAEL and all reliable LOAEL values from each study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. In a group of six individuals exposed to 2,000 ppm 1,4-dioxane vapors for 3 minutes in a 10 m^3 chamber, there were no complaints of nasal discomfort, but one out of four subjects exposed to 1,000 ppm for 5 minutes complained of constriction of the throat (Fairley et al. 1934); however, the exposure concentrations were not verified. Exposure of five subjects to about 278 ppm for a few minutes (unspecified) produced slight mucous membrane irritation, and 1,390 ppm caused a slight prickling in the nose, and scratchiness and dryness in the throat (Wirth and Klimmer 1936). Exposure to 300 ppm 1,4-dioxane for 15 minutes produced nose and throat irritation among a group of 12 volunteers (Silverman et al. 1946). At 200 ppm, the report does not indicate the presence or absence of symptoms, but considers the exposure acceptable. A 10-minute exposure to 1,600 ppm 1,4-dioxane produced slight nose and throat irritation that persisted throughout the test in a group of five individuals (Yant et al. 1930). In another experiment by the same investigators, exposure of the same five persons to 5,500 ppm 1,4-dioxane for 1 minute resulted in a burning sensation to the nose and throat (Yant et al. 1930). Exposure of four men to 50 ppm for 6 hours reportedly caused no adverse respiratory signs or alterations in respiratory function, assessed 24 hours and 2 weeks after exposure (Young et al. 1977); however, no data were provided in the study.

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Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL			Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)		
	ACUTE EXPOSURE							
	Death							
1	Rat (NS)	1 wk 5 d/wk 3 hr/d				5000	(3/3 deaths before a total of 16 hours of exposure)	Fairley et al. 1934
2	Rat (Wistar)	4 hr				14261 F	(4-hr LC50)	Pozzani et al. 1959
3	Mouse (NS)	1 wk 5 d/wk 3 hr/d				5000	(1/3 deaths after 3 hours of exposure)	Fairley et al. 1934
4	Gn Pig (NS)	1 wk 5 d/wk 3 hr/d				10000	(6/6 deaths before 7.5 hours of exposure)	Fairley et al. 1934
5	Gn Pig (NS)	10-540 min				30000	(death of majority of animals in 180 minutes)	Yant et al. 1930
6	Rabbit (NS)	1 wk 5 d/wk 3 hr/d				5000	(1/4 death after 16.5 hours of exposure)	Fairley et al. 1934

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	Systemic						
7	Human	5 min	Resp		1000 (1/4 throat constriction)		Fairley et al. 1934
8	Human	3 min	Resp	2000			Fairley et al. 1934
			Ocular	2000			
9	Human	15 min	Resp	200	300 (nose and throat irritation)		Silverman et al. 1946
			Ocular	200	300 (eye irritation)		
10	Human	10 min	Resp		1600 (slight nose and throat irritation)		Yant et al. 1930
			Ocular		1600 (slight eye irritation and lacrimation)		

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Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
11	Human	6 hr	Resp	50 M					Young et al. 1977
			Cardio	50 M					
			Hemato	50 M					
			Hepatic	50 M					
			Renal	50 M					
			Ocular		50[b] M	(eye irritation)			
12	Rat (CD)	4 hr	Hepatic		1000 M	(increased serum transaminases indicative of liver injury)			Drew et al. 1978
13	Gn Pig (NS)	10-540 min	Resp		1000	(immediate nasal irritation)	30000	(dyspnea, gasping, shallow breathing, hyperemia, congestion)	Yant et al. 1930
			Ocular	1000	2000	(eye irritation)			

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Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	Neurological						
14	Rat (Wistar)	2 wk 5 d/wk 4 hr/d		1500 F	3000 F (depressed avoidance response)		Goldberg et al. 1964
	INTERMEDIATE EXPOSURE						
	Death						
15	Rabbit (NS)	3-12 wk 5 d/wk 3 hr/d				2000 (1/4 death on week 4 of exposure)	Fairley et al. 1934
	Systemic						
16	Rat (NS)	3-12 wk 5 d/wk 3 hr/d	Resp	1000			Fairley et al. 1934
			Hepatic			1000 (hepatocyte degeneration)	
			Renal			1000 (renal cortex degeneration)	
17	Mouse (NS)	3-12 wk 5 d/wk 3 hr/d	Resp	1000			Fairley et al. 1934
			Hepatic			1000 (hepatocyte degeneration)	
			Renal			1000 (renal cortex degeneration)	

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form
18	Gn Pig (NS)	3-12 wk 5 d/wk 3 hr/d	Resp	5000				Fairley et al. 1934
			Hepatic			1000	(hepatocyte degeneration)	
			Renal			1000	(cortical cell degeneration)	
19	Rabbit (NS)	3-12 wk 5 d/wk 3 hr/d	Resp	1000				Fairley et al. 1934
			Hepatic			1000	(hepatocyte degeneration)	
			Renal			1000	(cortical cell degeneration)	

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Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Systemic						
20	Rat (Wistar)	2 yr 5 d/wk 7 hr/d	Resp	111			Torkelson et al. 1974
			Cardio	111			
			Gastro	111			
			Hemato	111			
			Hepatic	111[c]			
			Renal	111			
			Endocr	111			
			Dermal	111			
			Ocular	111			
			Bd Wt	111			
	Immuno/ Lymphoret						
21	Rat (Wistar)	2 yr 5 d/wk 7 hr/d		111			Torkelson et al. 1974
	Neurological						
22	Rat (Wistar)	2 yr 5 d/wk 7 hr/d		111			Torkelson et al. 1974

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form
					Less Serious (ppm)	Serious (ppm)	
	Reproductive						
23	Rat (Wistar)	2 yr 5 d/wk 7 hr/d		111			Torkelson et al. 1974

a The number corresponds to entries in figure 3-1.

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 2.0 ppm for 1,4-dioxane; the MRL was derived by dividing the LOAEL by an uncertainty factor of 30 (3 for a minimal LOAEL and 10 to protect sensitive populations).

c Used to derive a chronic-duration inhalation minimal risk level (MRL) of 1.0 ppm for 1,4-dioxane; the NOAEL was converted to a human equivalent concentration of 35.5 ppm by using a PBPK model. The MRL was derived by dividing the NOAEL-HEC by an uncertainty factor of 30 (3 for animal to human extrapolation using a dosimetric adjustment and 10 to protect sensitive populations). The chronic duration inhalation MRL was adopted also as intermediate-duration MRL.

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); F = Female; Gastro = gastrointestinal; Gn pig = guinea pig; hemato = hematological; hr = hour(s); LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); NOAEL = no-observed-adverse-effect level; NS = not specified; ppm = parts per million; Resp = respiratory; wk = week(s); yr = year(s)

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Figure 3-1. Levels of Significant Exposure to 1,4-Dioxane - Inhalation

Acute (≤14 days)

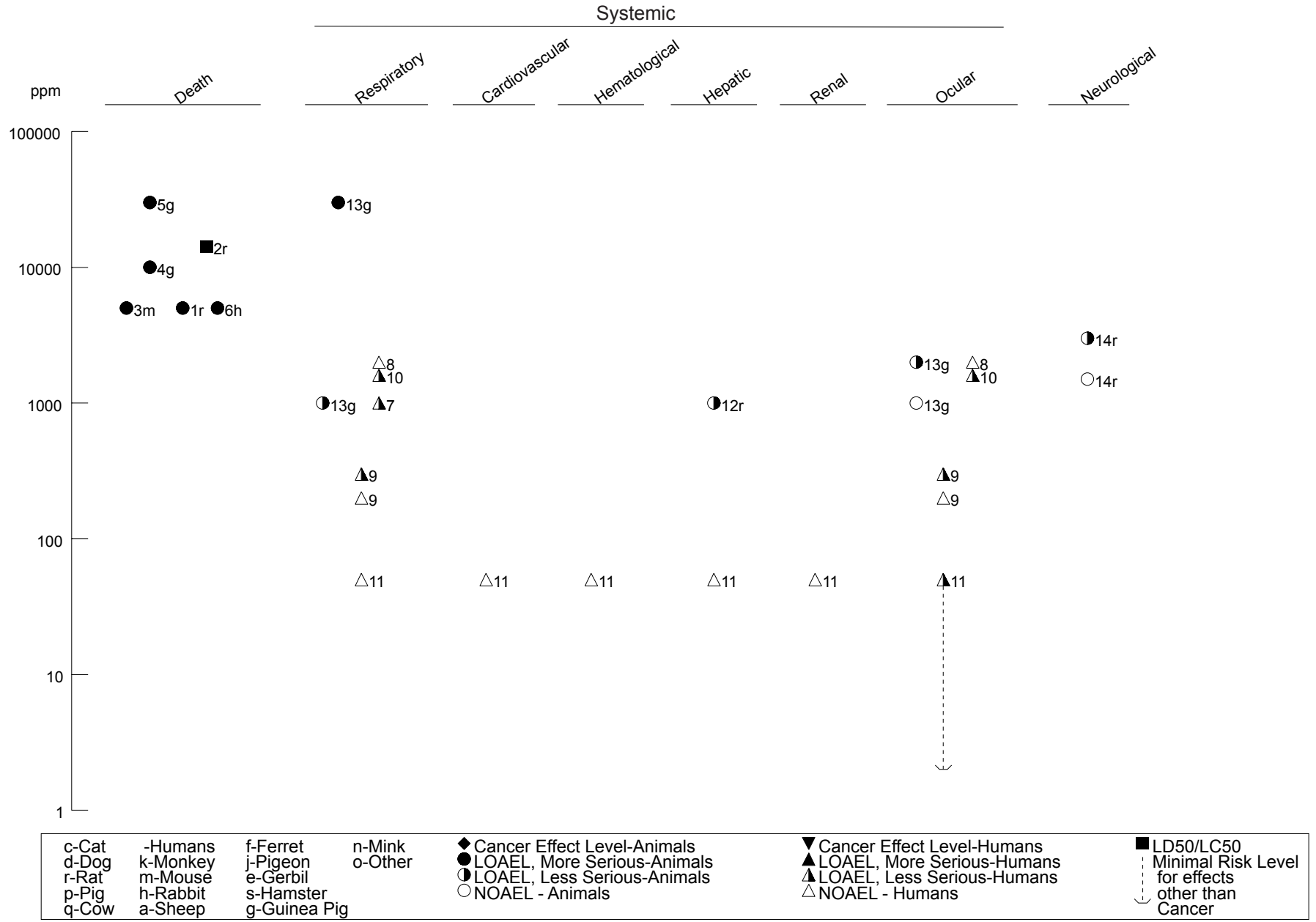

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Figure 3-1. Levels of Significant Exposure to 1,4-Dioxane - Inhalation (*Continued*)

Intermediate (15-364 days)

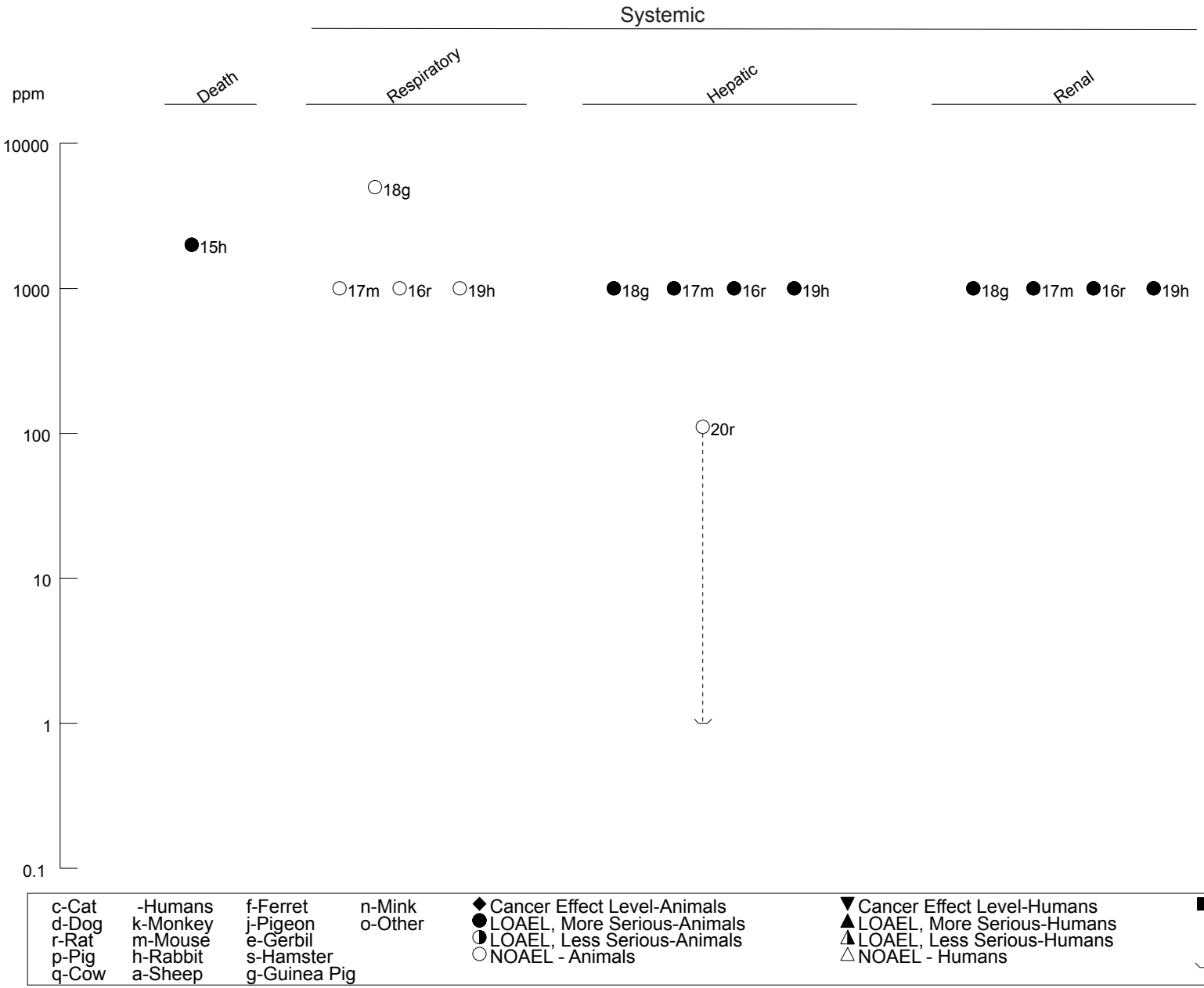

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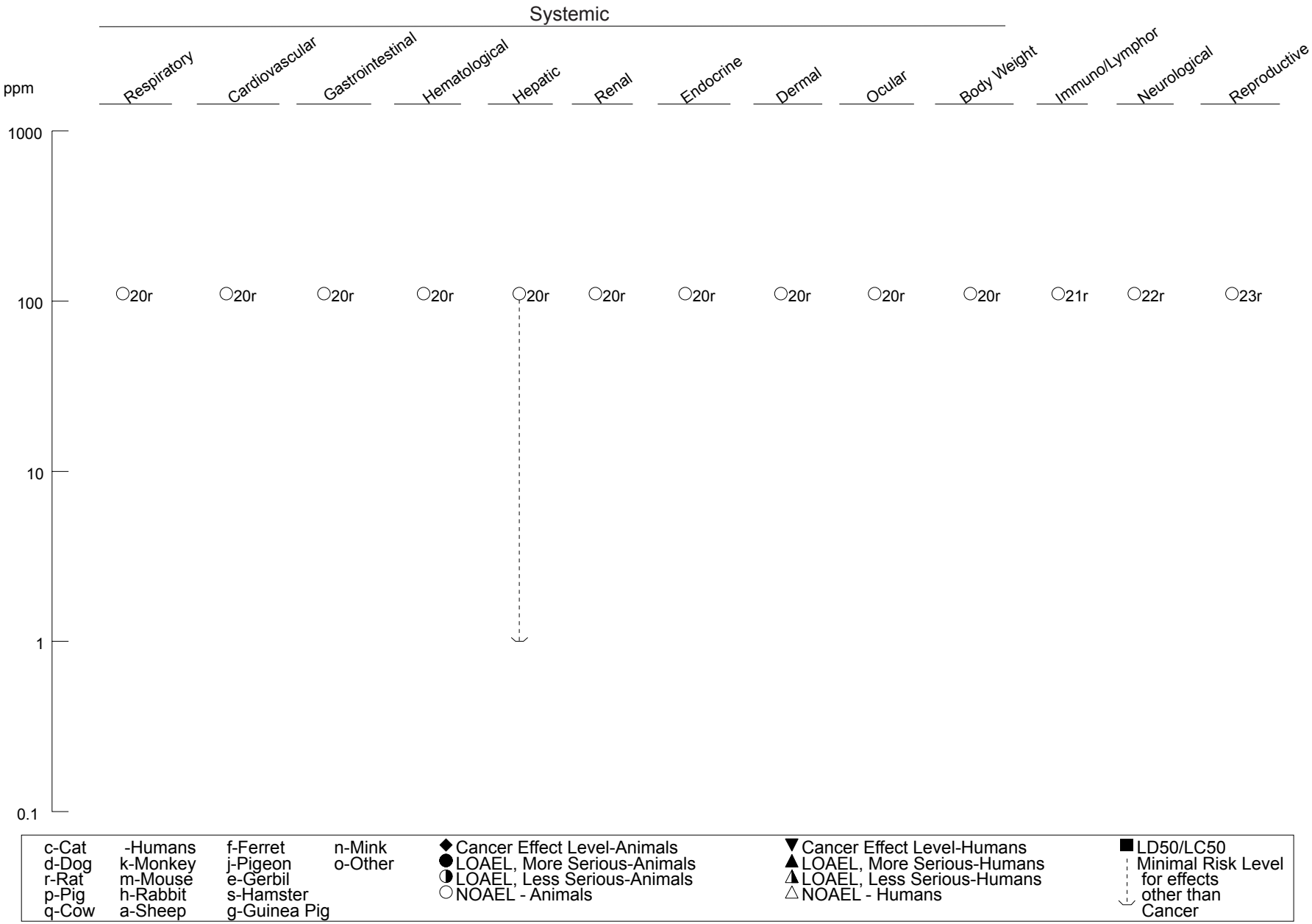
Figure 3-1. Levels of Significant Exposure to 1,4-Dioxane - Inhalation (*Continued*)

Chronic (=365 days)



In a study in guinea pigs exposed to 1,000–30,000 ppm 1,4-dioxane for periods ranging from 10 to 540 minutes, nasal irritation was evident almost immediately at all exposure levels (Yant et al. 1930). No respiratory changes were noticed with concentrations of up to 10,000 ppm for 480 minutes, but dyspnea and gasping occurred at 30,000 ppm for 45–116 minutes. The 30,000 ppm level caused death in the animals in about 180 minutes. Gross necropsy revealed exposure-duration-related hyperemia in the lungs. Surviving guinea pigs autopsied 8–10 days after exposure showed no gross pathological changes except for a few cases of hyperemic areas of congestion in the lungs. Exposure of rats and guinea pigs to acute lethal concentrations of 1,4-dioxane produced vascular congestion of the lungs (Fairley et al. 1934). No lung lesions were seen in rats, mice, or rabbits exposed to 1,000 ppm 1,4-dioxane for 3–12 weeks or in guinea pigs exposed to 5,000 ppm for the same duration (Fairley et al. 1934). In the 2-year inhalation study in rats by Torkelson et al. (1974), intermittent exposure to 111 ppm 1,4-dioxane caused no signs of nasal irritation, respiratory distress, or histopathologic alterations in the lungs and trachea of the animals.

Cardiovascular Effects. A study of four men exposed to 50 ppm 1,4-dioxane for 6 hours reported no abnormalities in the electrocardiograms (EKG) taken 24 hours and 2 weeks after exposure compared to EKGs taken prior to the study (Young et al. 1977); however, no data were provided in the study. High blood pressure was reported in subjects who eventually died following exposure to high amounts of 1,4-dioxane (Barber 1934; Johnstone 1959), but this may have been a non-specific response to a stressful condition or due to acute renal failure.

The only available information in animals is that no gross or histopathological alterations were observed in the heart from rats exposed to 111 ppm 1,4-dioxane for 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974).

Gastrointestinal Effects. Abdominal pain and vomiting were common features among subjects after exposure to high concentrations of 1,4-dioxane who eventually died (Barber 1934; Johnstone 1959). Barber (1934) suggested that the abdominal pain may have been due to stretching of the capsule of the liver and kidneys. No gross or histologic alterations were observed in the gastrointestinal tract from rats exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974).

Hematological Effects. A study of four male volunteers exposed to 50 ppm 1,4-dioxane for 6 hours reportedly did not show any significant effect of exposure on hematology parameters (Young et al. 1977); however, no data were provided in the study. Blood was collected prior to exposure and 24 hours and

2 weeks after exposure and subjected to a complete hematological analyses. Leukocytosis and eosinophilia were described in subjects who survived exposure to high concentrations of 1,4-dioxane described by Barber (1934). A cross-sectional study of 74 workers exposed to concentrations of 1,4-dioxane between 0.006 and 14.3 ppm for an average length of exposure of almost 25 years found no significant alterations in hemoglobin concentration, erythrocyte counts, and total and differential leukocyte counts among the subjects (Thiess et al. 1976).

In the 2-year inhalation study in rats by Torkelson et al. (1974), hematological parameters were measured in blood collected after 16 and 23 months of exposure. In this study, the rats were exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week. The specific hematological parameters measured were packed corpuscular volume, erythrocyte counts, hemoglobin concentration, and total and differential leukocyte counts. No toxicologically significant deviations from normal limits were found.

Hepatic Effects. Short-term exposure of humans to concentrations that eventually caused death produced serious liver damage. Barber (1934) described five lethal cases in which postmortem examination of the patients revealed an enlarged liver and centrilobular necrosis of the liver cells. Similar lesions were observed in a lethal case described by Johnstone (1959). Exposure of a group of four men to 50 ppm 1,4-dioxane for 6 hours produced no liver alterations as judged by standard clinical chemistry tests (although not specified) and triglyceride determination (Young et al. 1977). A cross sectional study of 74 workers exposed to concentrations of 1,4-dioxane between 0.006 and 14.3 ppm for an average length of exposure of almost 25 years found no conclusive evidence of serious liver damage (Thiess et al. 1976). Although 6 out of 24 current workers had elevated serum transaminase levels, all 6 were known as habitual alcohol drinkers.

Guinea pigs exposed to acute lethal concentrations of 1,4-dioxane had liver lesions ranging from cloudy swelling to areas of complete necrosis (Fairley et al. 1934). The effect of 1,4-dioxane on the levels of serum ALT, AST, ornithine carbamyl transferase (OCT), and glucose-6-phosphatase was studied in groups of male rats exposed to 0, 1,000, or 2,000 ppm 1,4-dioxane for 4 hours (Drew et al. 1978). The enzyme levels were used as indication of liver damage. Exposure to 1,4-dioxane markedly increased the activities (concentration-related) of AST, ALT, and OCT, particularly 48 hours after exposure. The activity of glucose-6-phosphatase was slightly increased 48 hours after exposure.

A study in which rats, mice, guinea pigs, and rabbits were exposed to 1,000 ppm (the lowest concentration tested) 3 hours/day, 5 days/week for 3–12 weeks reported hepatocyte degeneration of

varying severity in all of the species tested (Fairley et al. 1934). In the 2-year inhalation bioassay in rats exposed intermittently to 111 ppm 1,4-dioxane, there was no evidence of any exposure-related gross or microscopic liver alterations or alterations in serum AST and alkaline phosphatase activities (Torkelson et al. 1974). The NOAEL of 111 ppm was used to derive a chronic-duration inhalation MRL of 1 ppm for 1,4-dioxane.

Renal Effects. Swollen kidneys with hemorrhage was commonly seen in subjects who died following exposure to unknown amounts of 1,4-dioxane in the air described by Barber (1934). Microscopic examination showed hemorrhage around the glomeruli with some necrosis. Barber (1934) stated that in at least three of the five cases he described, kidney disease was the direct cause of death. In a fatal case described by Johnstone (1959), postmortem examination revealed necrosis in the kidney cortex with extensive interstitial hemorrhage. Exposure of a group of four men to 50 ppm 1,4-dioxane for 6 hours reportedly produced no kidney alterations as assessed by comparing serum creatinine values and urinalysis results obtained prior to exposure with results obtained 24 hours and 2 weeks after exposure (Young et al. 1977). No evidence of kidney damage was found in a cross-sectional study of 74 workers exposed to concentrations of 1,4-dioxane between 0.006 and 14.3 ppm for an average length of exposure of almost 25 years (Thiess et al. 1976).

Kidney lesions were commonly observed in rodents exposed to acute lethal concentrations of 1,4-dioxane (Fairley et al. 1934). Examination of rats, mice, guinea pigs, and rabbits exposed to 1,000 ppm 1,4-dioxane (the lowest concentration tested) 3 hours/day, 5 days/week for 3–12 weeks, showed varying degrees of kidney damage ranging from vascular congestion to renal cortex degeneration (Fairley et al. 1934). In general, exposure to higher concentrations increased the severity of the effects. In a 2-year inhalation study in rats exposed intermittently to 111 ppm 1,4-dioxane, there were no treatment-related gross or microscopic alterations in the kidneys or significant alterations in blood-urea nitrogen and total protein concentration (Torkelson et al. 1974).

Endocrine Effects. No gross or microscopic alterations were observed in the thyroid and pituitary glands from rats exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974). No further relevant information was located.

Dermal Effects. In the 2-year study in rats by Torkelson et al. (1974), the investigators indicated that intermittent exposure to a concentration of 111 ppm 1,4-dioxane in the air had no significant effect on skin condition; no microscopic examination of the skin was conducted. Had skin condition been affected,

it would have been most likely due to direct contact with the chemical rather than due to inhaled 1,4-dioxane.

Ocular Effects. In a group of six individuals exposed to 2,000 ppm 1,4-dioxane vapors for 3 minutes in a 10-m^3 chamber, there were no complaints of ocular discomfort (Fairley et al. 1934). Exposure to 300 ppm 1,4-dioxane for 15 minutes produced eye irritation among a group of 12 volunteers (Silverman et al. 1946). A 10-minute exposure to 1,600 ppm 1,4-dioxane produced slight eye irritation and lacrimation that persisted throughout the test in a groups of five individuals (Yant et al. 1930). In another experiment by the same investigators, exposure of the same five persons to 5,500 ppm 1,4-dioxane for 1 minute resulted in irritation of the eyes (Yant et al. 1930). Eye irritation throughout exposure was a frequent and the only complaint among four men exposed to 50 ppm for 6 hours in a study by Young et al. (1977). It is assumed that, in these cases, the irritation was caused by direct contact of the vapor with the eyes. The LOAEL of 50 ppm from the study by Young et al. (1977) was used to derive an acute-inhalation MRL of 2 ppm for 1,4-dioxane.

In a study in guinea pigs exposed to 1,000–30,000 ppm 1,4-dioxane for 10–540 minutes, eye irritation was observed at 2,000 ppm for 5 minutes and 3,000 ppm for 8 minutes, but not at 1,000 ppm for 480 minutes (Yant et al. 1930). No evidence of eye irritation was observed in rats exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years, but no histological examination of the eyes was performed (Torkelson et al. 1974).

Body Weight Effects. No significant effect on body weight gain was observed in rats exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974). No further relevant information was located.

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans following inhalation exposure to 1,4-dioxane. No gross or microscopic alterations were observed in lymph nodes or the spleen from rats exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974). This value is presented as a NOAEL for lymphoreticular effects in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

Edema of the brain was observed in three of the five fatal cases described by Barber (1934). However, as suggested by NIOSH (1977), these changes were probably terminal, rather than specific toxic effects of 1,4-dioxane. Also, brain damage, possibly secondary to anoxia and cerebral edema, was observed in a worker who died following combined inhalation and dermal exposure to a high amount of 1,4-dioxane (Johnstone 1959). Postmortem examination showed moderate perivascular widening of the brain and demyelination and partial loss of nerve fiber in small areas of the basal nuclei.

Exposure of rats to ≥3,000 ppm 1,4-dioxane 4 hours/day 5 days/week for 2 weeks resulted in depression of an avoidance response (Goldberg et al. 1964). The maximal effect was obtained after the second day of exposure. All of the effects on behavior were reversible. Exposure of rats to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years caused no significant gross or microscopic alterations in the brain (Torkelson et al. 1974). Although only the brain was evaluated by Torkelson et al. (1974), the rats were observed throughout the study for signs of toxicity, including activity and demeanor; therefore, the value of 111 ppm is presented as a NOAEL for neurological effects in Table 3-1 and is plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following inhalation exposure to 1,4-dioxane. No alterations were observed in the reproductive organs from male (testes) and female (ovaries, oviduct, uterus, and vagina) rats exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974). This NOAEL is presented in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or in animals following inhalation exposure to 1,4-dioxane.

3.2.1.7 Cancer

Limited information exists regarding exposure to 1,4-dioxane and cancer in humans. Thiess et al. (1976) conducted a cross-sectional study of 74 workers exposed to concentrations of 1,4-dioxane between 0.006 and 14.3 ppm for an average length of exposure of almost 25 years. Twelve deaths had been reported and two were attributed to cancer, but the overall death rate and the cancer death rate were not significantly different than expected rates. An additional occupational study of 165 workers exposed intermittently to concentrations of 1,4-dioxane between 0.1 and 17 ppm (the maximums ranged between 1.5 and 3.2 ppm) at least one month during a 21-year period to found no significant differences between observed and expected incidences of cancer (Buffler et al. 1978). However, the study was limited in power to detect an effect due to the small size of the cohort, low levels of exposure, and the relatively short exposures.

No evidence of carcinogenicity due to 1,4-dioxane was found in a study in Wistar rats (288/sex) in which the animals were exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974). A group of 192 rats/sex served as controls, and the evaluation included all major tissues and organs including the liver and nasal cavity.

3.2.2 Oral Exposure

3.2.2.1 Death

No reports of death in humans following oral exposure to 1,4-dioxane were found in the literature reviewed. Studies in animals have reported lethal doses in various species. Reported single dose LD_{50} values in rats include 5,346 mg/kg (Laug et al. 1939), 6,369 mg/kg (Pozzani et al. 1959), and 7,120 mg/kg (Smyth et al. 1941). Laug et al. (1939) also reported an LD_{50} of 5,852 mg/kg in mice and 4,033 mg/kg in guinea pigs. Two of 10 female rats dosed with 2,750 mg 1,4-dioxane/kg/day for 2 weeks in the drinking water died before the end of the study (JBRC 1998a). Smyth et al. (1941) calculated an LD_{50} of 3,150 mg/kg in guinea pigs, whereas de Navasquez (1935) reported 100% lethality in a group of five rabbits within 6 days of administration of a single dose of 2,068 mg of 1,4-dioxane/kg by gavage in water; a lower dose of 1,034 mg/kg was not lethal but produced narcolepsy, and doses of 207 mg/kg repeated at weekly intervals did not appear to affect the animals. All 10 female mice dosed with 3,230 mg 1,4-dioxane/kg/day in the drinking water died, and 9 of 10 males dosed with 3,630 mg/kg/day also died (JBRC 1998a). In a study using three dogs, consumption of approximately 327 mg

1,4-dioxane/kg/day via the drinking water killed one dog in 10 days, and consumption of approximately 375 mg/kg/day was lethal to an additional dog in 9 days (Schrenk and Yant 1936). Upon necropsy, common features in these animals were severe kidney and liver lesions consisting of cellular degeneration of the renal cortex and hemorrhages and vascular congestion in the kidneys and cellular degeneration in the liver. Because the dogs were allowed to drink the 1,4-dioxane solution only twice daily and no other source of water was available, dehydration may have played a role in their death.

In an intermediate-duration study, five of six rats dosed through drinking water that provided approximately 1,000 mg 1,4-dioxane/kg/day died between the 14th and 35th day of the study (Fairley et al. 1934). Necropsy of these animals revealed kidney and liver lesions. In a 2-year cancer bioassay in Sherman rats, significant early mortality beginning at about 2–4 months in the study was observed in males and females treated with 1,015 and 1,599 mg 1,4-dioxane/kg/day, respectively, in the drinking water (Kociba et al. 1974). Although the specific cause of death was not discussed, the investigators indicated that rats dying early showed degenerative changes in the liver and kidneys. Early mortality also was reported in other long-term studies in rats given ≥240 mg 1,4-dioxane/kg/day in the drinking water for 104–110 weeks (NCI 1978), or 398–514 mg/kg/day for 2 years (JBRC 1998c) and in mice treated similarly with ≥380 mg/kg/day for 90–104 weeks (NCI 1978) or ≥323 mg/kg/day for 2 years (JBRC 1998c). In the JBRC (1998c) study, early death in rats was attributed to liver and nasal cavity tumors and death in mice was attributed to liver tumors.

The LOAEL values for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, and body weight effects in humans after oral exposure to 1,4-dioxane.

The highest NOAEL and all reliable LOAEL values from each study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Rat (Fischer- 344)	2 wk ad libitum (W)				2750 F (2/10 deaths)	JBRC 1998a
2	Rat (NS)	12 d (W)				1034 (8/10 deaths within 12 days)	Kesten et al. 1939
3	Rat (NS)	once (G)				5346 (LD50)	Laug et al. 1939
4	Rat (Wistar)	once (G)				6369 F (LD50)	Pozzani et al. 1959
5	Rat (Wistar)	once (GW)				7120 (LD50)	Smyth et al. 1941
6	Mouse (B6C3F1)	2 wk ad libitum (W)				3230 F (10/10 deaths)	JBRC 1998a
7	Mouse (NS)	once (G)				5852 (LD50)	Laug et al. 1939

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL: Less Serious (mg/kg/day)	LOAEL: Serious (mg/kg/day)	Reference Chemical Form
8	Gn Pig (NS)	once (G)				4033 (LD50)	Laug et al. 1939
9	Gn Pig (NS)	once (GW)				3150 (LD50)	Smyth et al. 1941
10	Rabbit (NS)	once (GW)				2068 (5/5 deaths in 2-6 days)	De Navasquez 1935
	Systemic						
11	Rat (Fischer- 344)	2 wk ad libitum (W)	Resp	370[b] M	1010 M (nuclear enlargement of olfactory epithelium)		JBRC 1998a
			Hepatic	1040 F	2750 F (hepatocyte swelling and vacuolation)		
			Renal	1040 F	2750 F (hydropic change in proximal tubule)		
			Bd Wt	1040 F		2750 F (24% reduced body weight gain)	

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
12	Rat (NS)	12 d (W)	Hepatic		1034 (unspecified liver abnormalities)		Kesten et al. 1939
			Renal			1034 (kidney degeneration)	
13	Rat (Sprague-Dawley)	once (GW)	Hepatic	1000 M			Stott et al. 1981
14	Mouse (B6C3F1)	2 wk ad libitum (W)	Hepatic	1380 M	2550 M (swelling of central area)		JBRC 1998a
			Bd Wt	1380 M	2550 M (swelling of central area)		
	Neurological						
15	Rat (Fischer- 344)	2 wk ad libitum (W)		1040 F		2750 F (vacuolar changes in the brain)	JBRC 1998a
16	Rabbit (NS)	once (GW)		207		1034 (narcolepsy, slow gate, ataxia)	De Navasquez 1935

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
17	Rabbit (NS)	once (G)		1760	4400	(staggering)	6600	(narcosis)	Knoefel 1935
	Developmental								
18	Rat (Sprague-Dawley)	9 d Gd 6-15 (GW)		516	1033	(decreased fetal weight; reduced sternum ossification)			Giavini et al. 1985
	INTERMEDIATE EXPOSURE								
	Death								
19	Rat (NS)	34 d ad libitum (W)					1000	(5/6 deaths before the 35th day)	Fairley et al. 1934
20	Rat (Sherman)	2-4 mo ad libitum (W)					1015 M	(significant early mortality beginning at 2 months in the study)	Kociba et al. 1974
	Systemic								
21	Rat (NS)	34 d ad libitum (W)	Gastro		1428	(gastroenteritis)			Fairley et al. 1934
			Hepatic				1428	(hepatocyte degeneration)	
			Renal				1428	(renal cortex degeneration)	

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL: Less Serious (mg/kg/day)	LOAEL: Serious (mg/kg/day)	Reference Chemical Form
22	Rat (Fischer- 344)	13 wk ad libitum (W)	Resp	60 M	150 M (nuclear enlargement of respiratory epithelium)		JBRC 1998b
			Cardio	2020 F			
			Gastro	2020 F			
			Hemato	760 M	1900 M (increased red blood cell, hemoglobin, hematocrit, neutrophils)		
			Musc/skel	2020 F			
			Hepatic	60[c] M	150 M (swelling in central area)		
			Renal	330 M	760 M (nuclear enlargement of proximal tubule)		
				100[d] F	200[d] F (increased kidney weight)		
			Endocr	2020 F			
			Dermal	2020 F			
			Ocular	2020 F			
			Bd Wt	430 M	870 F (12% reduction in weight gain)	2020 F (21% reduction in body weight gain)	

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
23	Rat (Sprague-Dawley)	7 wk 5 d/wk (GW)	Hepatic	100 M	1000 M (fatty vacuoles in cytoplasm of hepatocytes)		Lundberg et al. 1987
24	Rat (Sprague-Dawley)	11 wk ad libitum (W)	Hepatic	10 M	1000 M (minimal hepatocellular swelling)		Stott et al. 1981
			Bd Wt	1000 M			
25	Mouse (NS)	67 d ad libitum (W)	Hepatic			2916 (hepatocyte degeneration)	Fairley et al. 1934
			Renal			2916 (cell degeneration in renal cortex)	

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
26	Mouse (B6C3F1)	13 wk ad libitum (W)	Resp	170 F	410 F (nuclear enlargement of bronchial epithelium)		JBRC 1998b
			Cardio	2700 F			
			Gastro	2700 F			
			Hemato	920 M	1830 M (increase red blood cell, hemoglobin, hematocrit, corpuscular volume)		
			Musc/skel	2700 F			
			Hepatic	260 M	580 M (single cell necrosis and swelling of central area)		
			Renal	1710 F	2700 F (increased relative kidney weight)		
			Endocr	2700 F			
			Dermal	2700 F			
			Ocular	2700 F			
			Bd Wt	920[d] M		1830 M (29% reduced body weight gain)	
				2700 F			

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Immuno/ Lymphoret						
27	Rat (Fischer- 344)	13 wk ad libitum (W)		2020 F			JBRC 1998b
28	Mouse (B6C3F1)	13 wk ad libitum (W)		2700 F			JBRC 1998b
	Neurological						
29	Rat (Fischer- 344)	13 wk ad libitum (W)		760 M		1900 M (vacuolar changes in the brain)	JBRC 1998b
30	Mouse (B6C3F1)	13 wk ad libitum (W)		2700 F			JBRC 1998b
	Reproductive						
31	Rat (Fischer- 344)	13 wk ad libitum (W)		1900 M[d] 2020 F			JBRC 1998b

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
32	Mouse (B6C3F1)	13 wk ad libitum (W)		1830[d] M 2700 F			JBRC 1998b
	CHRONIC EXPOSURE						
	Death						
33	Rat (Fischer- 344)	2 yr ad libitum (W)				398 M (increased early mortality)	JBRC 1998c
34	Rat (Osborne-Mendel)	110 wk ad libitum (W)				240 M (early mortality)	NCI 1978
35	Mouse (B6C3F1)	2 yr ad libitum (W)				323 F (increased early mortality)	JBRC 1998c
36	Mouse (B6C3F1)	90 wk ad libitum (W)				380 F (early mortality)	NCI 1978

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Systemic						
37	Rat (Wistar)	452 d ad libitum (W)	Renal		584 M (glomerulonephritis)		Argus et al. 1965

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
38	Rat (Fischer- 344)	2 yr ad libitum (W)	Resp	21 F	103 F (nuclear enlargement of olfactory epithelium)		JBRC 1998c
			Cardio	514 F			
			Gastro	514 F			
			Hemato	16 M	81 M (decreased erythocytes, hemoglobin, hematocrit)		
			Musc/skel	514 F			
			Hepatic	16 M	81 M (liver hyperplasia, spongiosis, clear cell foci)		
			Renal	21 F	103 F (blood in the urine; nuclear enlargement of proximal tubule)		
			Endocr	514 F			
			Dermal	514 F			
			Ocular	514 F			
			Bd Wt	81 M	398 M (10% reduced body weight gain)		

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
39	Rat (Sherman)	716 d ad libitum (W)	Resp	1599 F			Kociba et al. 1974
			Cardio	1599 F			
			Gastro	1599 F			
			Hemato	1599 F			
			Hepatic	9.6[e] M			
				94 M			
			Renal	9.6 M	94 M (degeneration and necrosis of tubular epithelium)		
			Endocr	1599 F			
			Bd Wt	94 M	1015 M (>10% reduced weight gain)		

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
40	Rat (Osborne-Mendel)	110 wk ad libitum (W)	Resp		240 M (increased incidence of pneumonia)		NCI 1978
			Cardio	640 F			
			Gastro		240 M (stomach ulcers)		
			Musc/skel	640 F			
			Hepatic	240 M	350 F (hepatocytomegaly)		
			Renal			240 M (cortical tubular degeneration)	
			Endocr	640 F			
			Dermal	640 F			
			Bd Wt	240 M	530 M (reduced body weight gain, unquantified)		

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
41	Mouse (B6C3F1)	2 yr ad libitum (W)	Resp	66 M	251 M (nuclear enlargement of olfactory epithelium in nasal cavity)		JBRC 1998c
			Cardio	1066 F			
			Gastro	1066 F			
			Hemato	77 F	323 F (reduced blood platelets)		
			Musc/skel	1066 F			
			Hepatic	66 M	251 M (increased serum AST, ALT, AP, and LDH activities)		
			Renal	77 F	323 F (increased protein, glucose, and blood in urine)		
			Endocr	1066 F			
			Dermal	1066 F			
			Ocular	1066 F			
			Bd Wt	251 M	323 F (15% reduced body weight gain)	768 M (43% reduced body weight gain)	
				77 F[d]			

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL: Less Serious (mg/kg/day)	LOAEL: Serious (mg/kg/day)	Reference Chemical Form
42	Mouse (B6C3F1)	90 wk ad libitum (W)	Resp		380 F (increased incidence of pneumonia)		NCI 1978
			Cardio	860 F			
			Gastro	860 F			
			Musc/skel	860 F			
			Hepatic	860 F			
			Renal	860 F			
			Endocr	860 F			
			Dermal	860 F			
			Bd Wt	830 M 380 F[d]	860 F (decreased body weight gain, unquantified)		
	Immuno/ Lymphoret						
43	Rat (Fischer- 344)	2 yr ad libitum (W)		514 F			JBRC 1998c

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
44	Rat (Sherman)	716 d ad libitum (W)		1599 F			Kociba et al. 1974
45	Rat (Osborne-Mendel)	110 wk ad libitum (W)		640 F			NCI 1978
46	Mouse (B6C3F1)	2 yr ad libitum (W)		1066 F			JBRC 1998c
47	Mouse (B6C3F1)	90 wk ad libitum (W)		860 F			NCI 1978
	Neurological						
48	Rat (Fischer- 344)	2 yr ad libitum (W)		514 F			JBRC 1998c
49	Rat (Sherman)	716 d ad libitum (W)		1599 F			Kociba et al. 1974

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
50	Rat (Osborne-Mendel)	110 wk ad libitum (W)		640 F			NCI 1978
51	Mouse (B6C3F1)	2 yr ad libitum (W)		1066 F			JBRC 1998c
52	Mouse (B6C3F1)	90 wk ad libitum (W)		860 F			NCI 1978
	Reproductive						
53	Rat (Fischer- 344)	2 yr ad libitum (W)		514 F			JBRC 1998c
54	Rat (Sherman)	716 d ad libitum (W)		1599 F			Kociba et al. 1974
55	Rat (Osborne-Mendel)	110 wk ad libitum (W)		640 F			NCI 1978

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
56	Mouse (B6C3F1)	2 yr ad libitum (W)		1066 F				JBRC 1998c
57	Mouse (B6C3F1)	90 wk ad libitum (W)		860 F				NCI 1978
	Cancer							
58	Rat (Wistar)	452 d ad libitum (W)				584 M	(CEL: liver tumors)	Argus et al. 1965
59	Rat (Fischer- 344)	2 yr ad libitum (W)				398 M	(CEL: combined squamous cell carcinoma, sarcoma, rhabdomyosarcoma, esthesioneuroepithelioma of nasal cavity; hepatocallular carcinoma and sarcoma of the liver; mesothelioma of the peritoneum)	JBRC 1998c

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
60	Rat (Sherman)	716 d ad libitum (W)				1015 M (CEL: hepatocellular carcinomas)	Kociba et al. 1974
61	Rat (Osborne-Mendel)	110 wk ad libitum (W)				350 F (CEL:hepatocellular carcinomas) 240 (CEL: nasal carcinomas in both sexes)	NCI 1978
62	Mouse (B6C3F1)	2 yr ad libitum (W)				77 F (CEL: hepatocellular adenomas and carcinomas)	JBRC 1998c
63	Mouse (B6C3F1)	90 wk ad libitum (W)				380 F (CEL: increased incidence of hepatocellular carcinomas and adenomas)	NCI 1978

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Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
64	Gn Pig (NS)	23 mo ad libitum (W)				1014 M (CEL: increased incidence of hepatomas)	Hoch-Ligeti and Argus 1970

a The number corresponds to entries in figure 3-2.

b Used to derive an acute-duration oral minimal risk level (MRL) of 4.0 mg/kg/day for 1,4-dioxane; the MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 animal to human extrapolation and 10 to protect sensitive populations).

c Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.6 mg/kg/day for 1,4-dioxane; the MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 animal to human extrapolation and 10 to protect sensitive populations).

d Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

e Used to derive a chronic-duration oral minimal risk level (MRL) of 0.1 mg/kg/day for 1,4-dioxane; the MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 animal to human extrapolation and 10 to protect sensitive populations)

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); endocr = endocrine; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; (GW) = gavage in water; hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; (W) = drinking water; wk = week(s)

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Figure 3-2. Levels of Significant Exposure to 1,4-Dioxane - Oral

Acute (≤14 days)

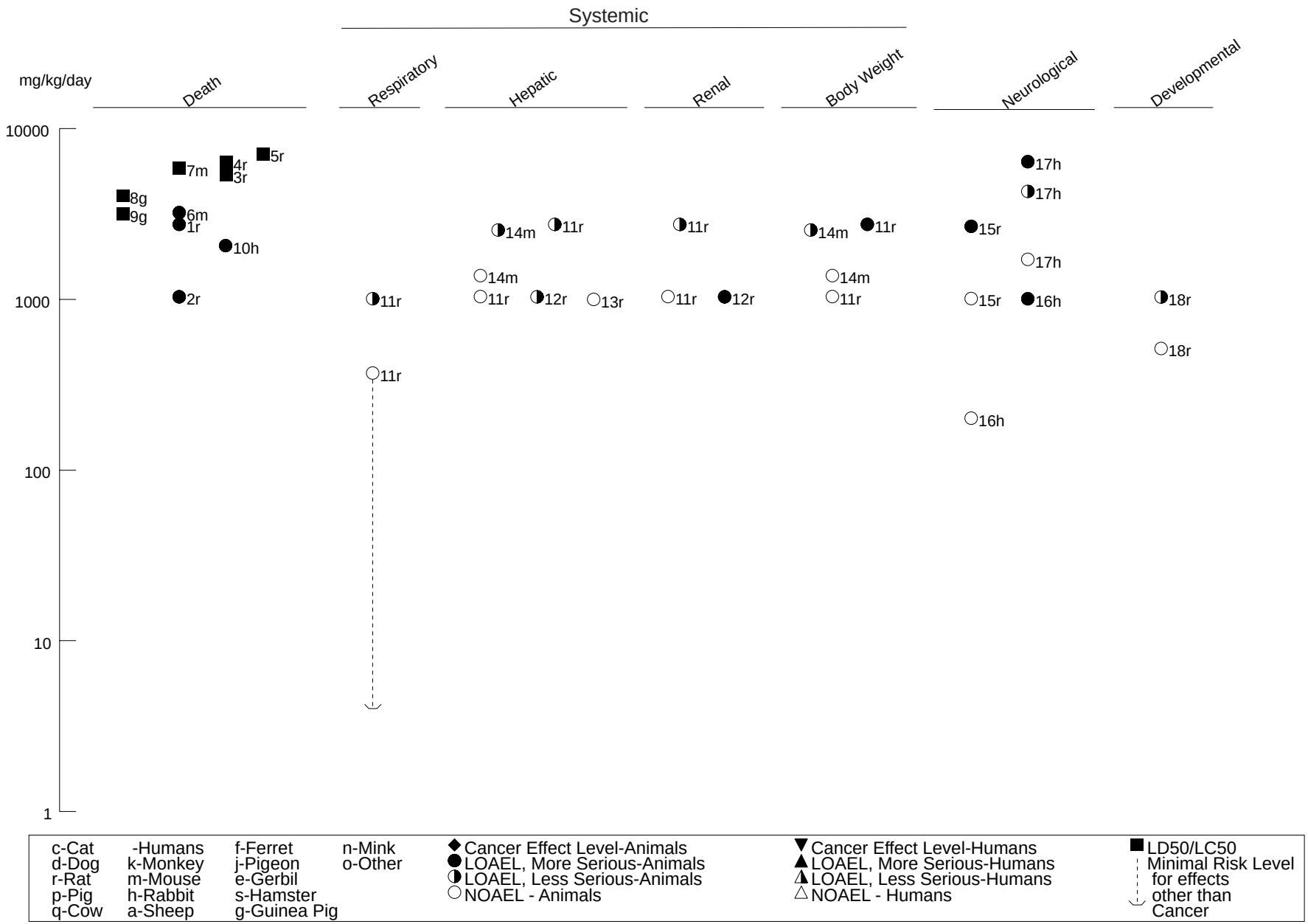

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Figure 3-2. Levels of Significant Exposure to 1,4-Dioxane - Oral (*Continued*)

Intermediate (15-364 days)

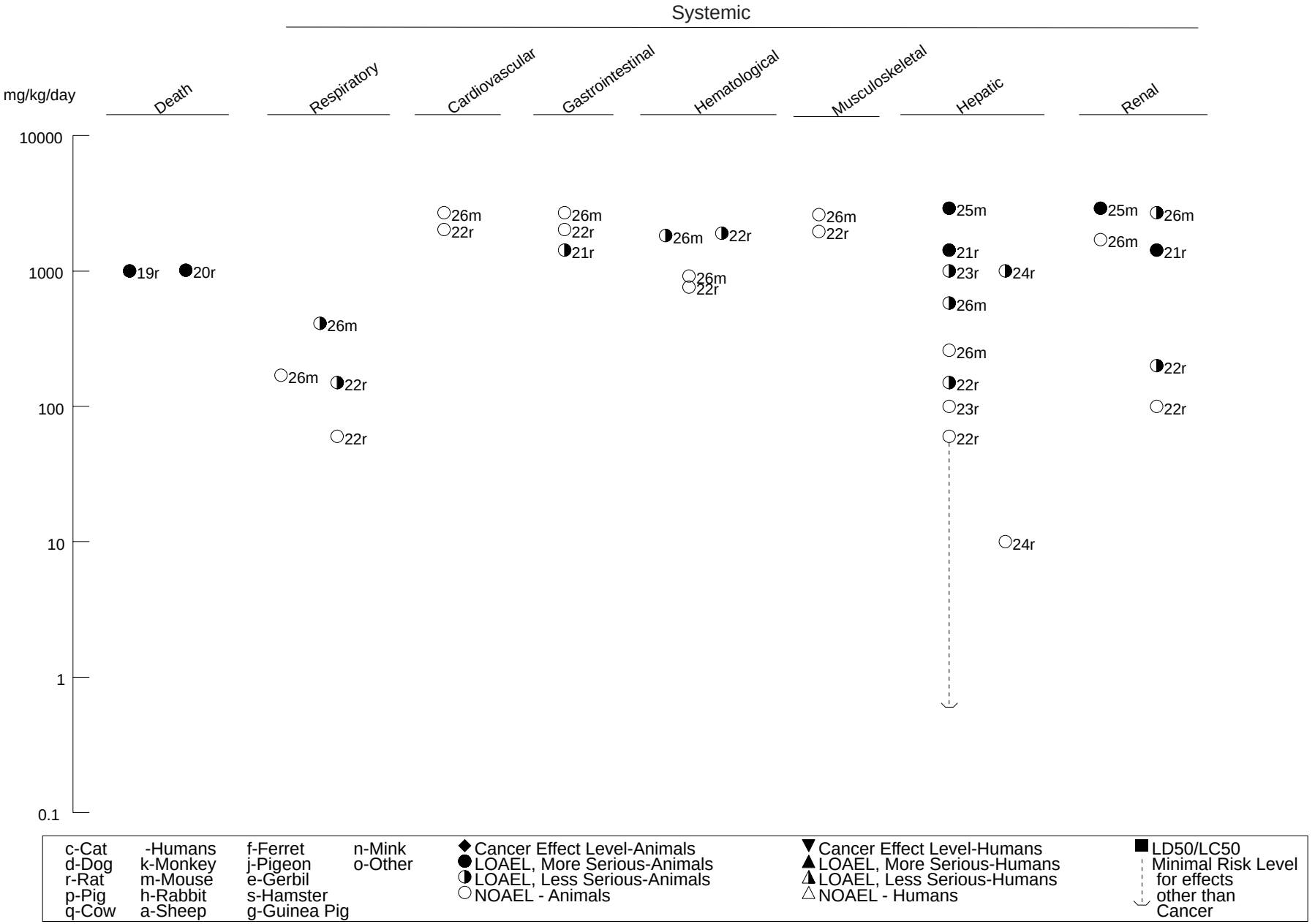

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Figure 3-2. Levels of Significant Exposure to 1,4-Dioxane - Oral (*Continued*)

Intermediate (15-364 days)

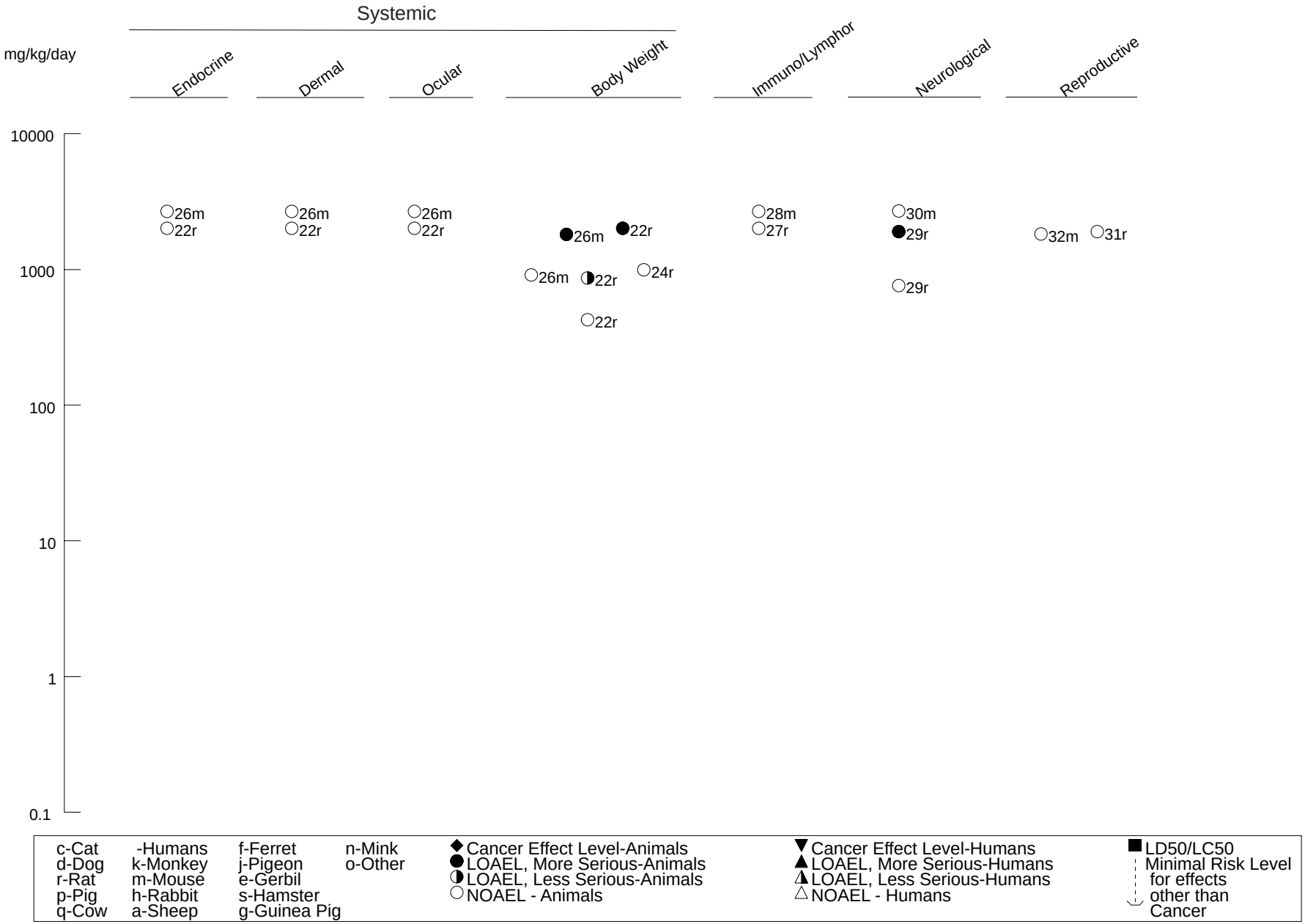

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Figure 3-2. Levels of Significant Exposure to 1,4-Dioxane - Oral (*Continued*)

Chronic (≥365 days)

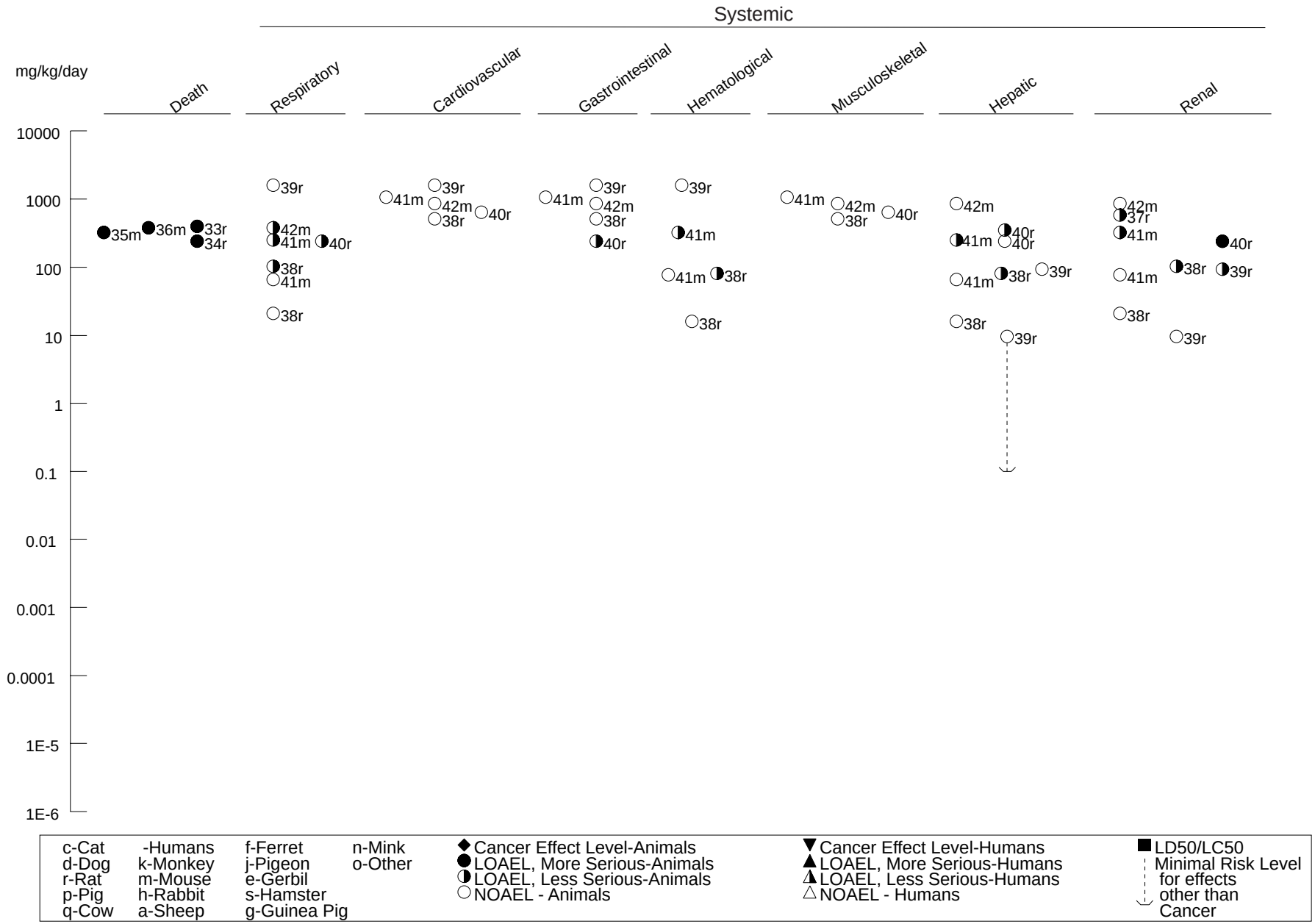

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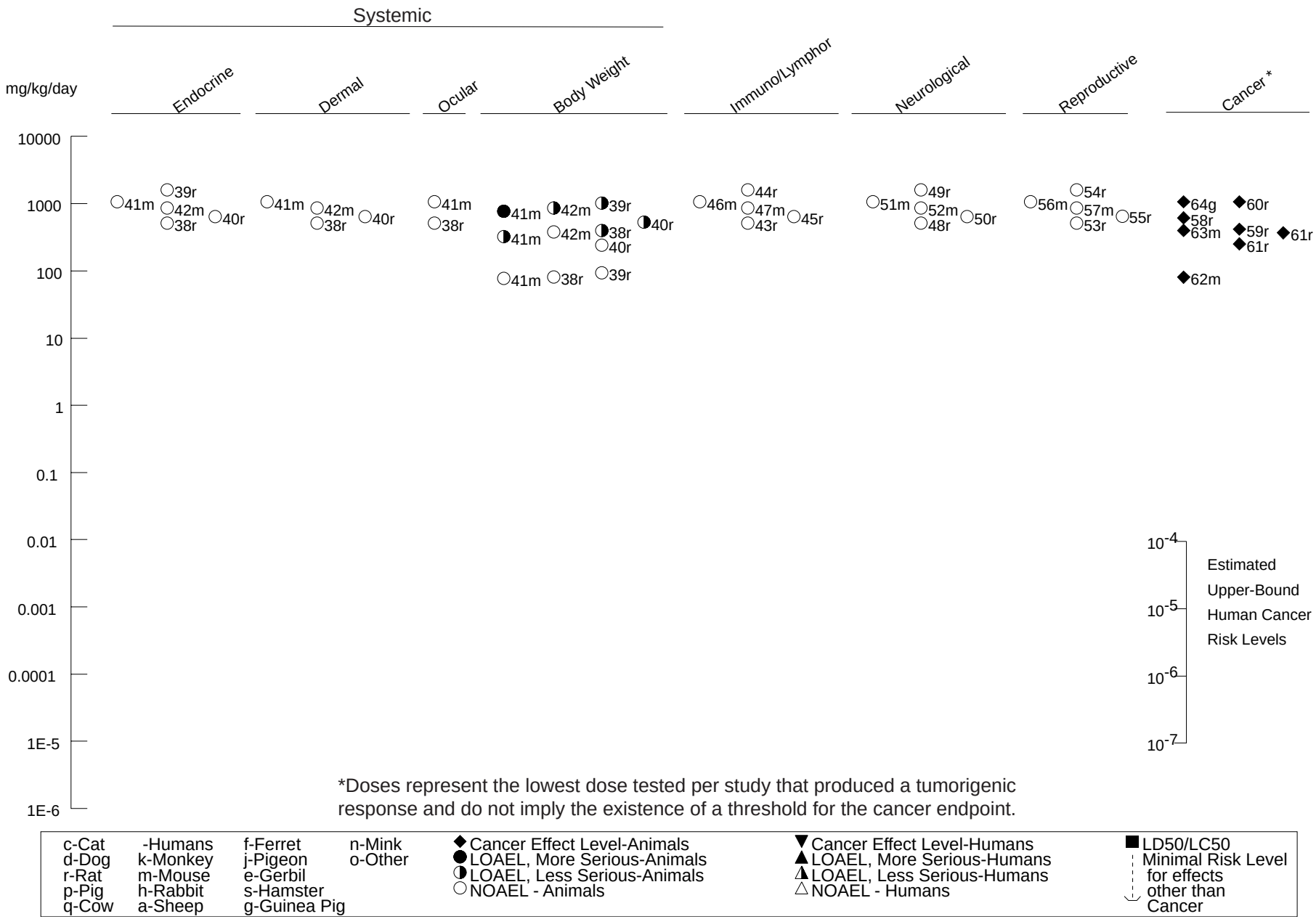
Figure 3-2. Levels of Significant Exposure to 1,4-Dioxane - Oral (*Continued*)

Chronic (≥365 days)



Respiratory Effects. Information exists regarding nasal and respiratory effects in animals after oral exposure to 1,4-dioxane. Nuclear enlargement of the olfactory epithelium was observed in male and female F344/DuCrj rats dosed with 1,010 and 1,040 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 2 weeks (JBRC 1998a); the respective NOAELs were 370 and 400 mg/kg/day. The same type of lesions were observed in male and female rats treated with 150 and 200 mg 1,4-dioxane/kg/day, respectively, for 13 weeks; the respective NOAELs were 60 and 100 mg/kg/day (JBRC 1998b). The nasal effects in rats described by JBRC (1998a) were used to derive an acute-duration oral MRL of 4 mg/kg/day for 1,4-dioxane. Male and female Crj:BDF_1 mice dosed with 580 and 410 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks showed nuclear enlargement of the bronchial epithelium; higher doses also involved the nasal cavity, trachea, and lungs (JBRC 1998b); the respective NOAELs were 260 and 170 mg/kg/day. No histopathologic changes were observed in the lungs and nasal turbinates from Sherman rats dosed with up to 1,599 mg 1,4-dioxane/kg/day in the drinking water for 716 days (Kociba et al. 1974). No significant non-neoplastic lesions were seen in the lungs and trachea from Osborne-Mendel rats dosed with up to 640 mg 1,4-dioxane/kg/day via drinking water for 110 weeks (NCI 1978). However, an increased incidence of pneumonia was seen in treated males and females, although the incidence was not dose-related. The investigators speculated that the development of nasal carcinomas might have been a contributing factor (NCI 1978). Female Fischer 344 rats dosed with approximately 103 mg 1,4-dioxane/kg/day, also in the drinking water, for 104 weeks showed a nuclear enlargement of the olfactory epithelium in the nasal cavity, the NOAEL was 21 mg/kg/day (JBRC 1998c). In males, nasal cavity lesions were observed at 398 mg/kg/day, and the NOAEL in males was 81 mg/kg/day.

In B6C3F_1 mice treated with 1,4-dioxane in the drinking water for 90 weeks, there was a dose-related increase in the incidence of pneumonia in males and females and of rhinitis in females (NCI 1978); males were dosed with 720 or 830 mg/kg/day and females were dosed with 380 or 860 mg/kg/day. Examination of the lungs and trachea did not reveal any other treatment-related non-neoplastic alterations. Male Crj:BDF_1 mice dosed with 251 mg 1,4-dioxane/kg/day in the drinking water for 2 years showed nuclear enlargement of olfactory epithelium in the nasal cavity; the NOAEL was 66 mg/kg/day (JBRC 1998c). Higher doses also involved the tracheal and bronchial epithelium. In females, nasal and bronchial alterations were observed at ≥323 mg/kg/day.

Cardiovascular Effects. No gross or histological alterations were observed in the heart from rats and mice dosed with up to 2,020 and 2,700 mg 1,4-dioxane/kg/day, respectively, in the drinking water for

13 weeks (JBRC 1998b). Also, no gross or histological alterations were reported in the heart from rats dosed with up to 1,599 mg 1,4-dioxane/kg/day in the drinking water for 2 years (JBRC 1998c; Kociba et al. 1974; NCI 1978) or in mice dosed with up to 860 mg/kg/day for 90 weeks (NCI 1978) or 1,066 mg/kg/day for 2 years (JBRC 1998c).

Gastrointestinal Effects. Hemorrhage of the stomach was reported in rats, mice, guinea pigs, and dogs administered acute lethal doses of 1,4-dioxane by gavage (Laug et al. 1939; Schrenk and Yant 1936; Smyth et al. 1941). Gastroenteritis was also reported in rats that died after drinking water that provided approximately 1,428 mg 1,4-dioxane/kg/day for up to 34 days (Fairley et al. 1934). No gross or histological alterations were observed in the gastrointestinal tract from rats and mice dosed with up to 2,020 and 2,700 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (JBRC 1998b), In chronic-duration studies, no gastrointestinal alterations were reported in Sherman rats dosed with up to 1,599 mg 1,4-dioxane/kg/day (Kociba et al. 1974), in F344/DuCrj rats dosed with up to 514 mg/kg/day (JBRC 1998c), in $B6C3F_1$ mice treated with up to 860 mg/kg/day (NCI 1978), or in Crj:BDF_1 mice dosed with up to 1,066 mg/kg/day (JBRC 1998c). However, male Osborne-Mendel rats treated with ≥240 mg/kg/day for 110 weeks developed stomach ulcers; no such lesions were seen in control males or in female rats (NCI 1978).

Hematological Effects. Hematological changes consisting of increased red blood cell counts, hemoglobin, and hematocrit were reported in F344/DuCrj male rats dosed with 1,900 mg 1,4-dioxane/kg/day in the drinking water for 13 weeks; no significant changes occurred at 760 mg/kg/day (JBRC 1998b). In females, there was a decrease in mean corpuscular volume and platelet concentration at 2,020 mg/kg/day. The same authors reported that male Crj:BDF_1 mice treated with 1,830 mg 1,4-dioxane/kg/day had increased red blood cell counts, hemoglobin, hematocrit, and mean corpuscular volume; the NOAEL was 920 mg/kg/day. Female mice only showed an increase in mean corpuscular volume at ≥1,710 mg/kg/day. Decreased red blood cell counts, hemoglobin, and hematocrit was reported in male and female F344/DuCrj rats treated with 81 and 514 mg 1,4-dioxane/kg/day, respectively, for 2 years in the drinking water (JBRC 1998c). The respective NOAELs were 16 and 103 mg/kg/day. However, Sherman rats showed no significant deviations from normality in hematological parameters in another 2-year study (Kociba et al. 1974). In the latter study, the rats were dosed with up to 1,599 mg 1,4-dioxane/kg/day in the drinking water; blood samples were collected during the 4^{th}, 6^{th}, 12^{th}, and 18^{th} month and at termination, and analyzed for packed cell volume, total erythrocyte counts, hemoglobin concentration, and total and differential white blood cell counts.

Male Crj:BDF_1 mice dosed with 768 mg 1,4-dioxane/kg/day in the drinking water for 2 years had increased red blood cell counts, hemoglobin, and hematocrit, whereas females exhibited a significant reduction in platelet concentration at ≥323 mg/kg/day (JBRC 1998c). The respective hematological NOAELs were 251 and 77 mg/kg/day.

Musculoskeletal Effects. No gross or histological alterations were observed in bone and skeletal muscle (neither specified) from rats and mice dosed with up to 2,020 and 2,700 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (JBRC 1998b), or in rats and mice dosed with up to 514 and 1,066 mg 1,4-dioxane/kg/day, respectively, for 2 years (JBRC 1998c). Similarly, no histopathologic alterations were observed in skeletal muscle from rats dosed with up to 640 mg 1,4-dioxane/kg/day for 110 weeks or in mice treated with up to 860 mg/kg/day for 90 weeks (NCI 1978).

Hepatic Effects. Acute oral doses of 1,4-dioxane that caused lethality in rats, mice, rabbits, guinea pigs, and dogs (see Section 3.2.2.1) induced varying degrees of liver damage, including liver congestion and degeneration (de Navasquez 1935; Kesten et al. 1939; Laug et al. 1939; Schrenk and Yant 1936; Smyth et al. 1941). In general, single doses that caused death were higher than 2,000 mg/kg. A single dose of 1,000 mg/kg administered to rats, and that did not cause death, produced no histopathologic alterations in the liver (Stott et al. 1981). Hepatocyte swelling and vacuolation of the central area were reported in the liver from F344/DuCrj rats dosed with 2,750–2,960 mg 1,4-dioxane/kg/day for 2 weeks in the drinking water (JBRC 1998a), but no significant liver alterations were seen at 1,010–1,040 mg/kg/day. Crj:BDF_1 mice treated in the same manner with 2,550–3,230 mg 1,4-dioxane/kg/day showed single cell necrosis and swelling of the central area; no significant alterations were reported at 1,380–1,780 mg/kg/day (JRBC 1998a). Repeated administration of doses of approximately 1,428 mg 1,4-dioxane/kg/day in the drinking water for 34 days was lethal to rats and examination of the animals showed liver congestion and hepatocyte degeneration (Fairley et al. 1934). The same types of liver lesions were seen in mice treated in the same manner with approximately 2,916 mg 1,4-dioxane/kg/day; in this experiment, the mice survived up to day 67, at which time they were sacrificed (Fairley et al. 1934). Repeated dosing of rats with 1,000 mg 1,4-dioxane/kg/day for 7 or 11 weeks produced hepatocyte swelling and histopathology (Stott et al. 1981) and fatty vacuoles in the hepatocytes (Lundberg et al. 1987). Male F344/DuCrj rats dosed with ≥150 mg 1,4-dioxane/kg/day for 13 weeks in the drinking water showed swelling of the central area (JBRC 1998b); the NOAEL was 60 mg/kg/day. Higher doses also produced vacuolar changes and granulation, and changes in clinical chemistry parameters indicative of liver toxicity. In female rats, granulation was evident at 430 mg/kg/day and hepatocyte swelling at 870 mg/kg/day. The findings from the JBRC (1998b) study in rats were used to derive an intermediate-

duration oral MRL of 0.6 mg/kg/day for 1,4-dioxane using a NOAEL of 60 mg/kg/day for male rats. In Crj:BDF_1 mice treated in the same manner, doses of 580–920 mg 1,4-dioxane/kg/day caused single cell necrosis and swelling in the central area, but doses ≤410 mg/kg/day were without significant effect (JBRC 1998b). Changes in clinical chemistry parameters suggestive of liver damage were reported also in mice dosed with ≥580 mg 1,4-dioxane/kg/day (JBRC 1998b). In the 2-year bioassay by Kociba et al. (1974) in Sherman rats, significant early deaths occurred with doses between 1,015 and 1,599 mg/kg/day beginning at about 2–4 months in the study, and the authors indicated that these rats exhibited degenerative changes in the liver, although is was not made clear whether these changes along with renal lesions were the cause of death. Rats treated chronically with 1,4-dioxane in the drinking water (≥94 mg/kg/day for males, ≥148 mg/kg/day for females) had liver lesions consisting of various degrees of hepatocellular degeneration and necrosis and evidence of hepatic regeneration, as indicated by hepatocellular hyperplastic nodule formation (Kociba et al. 1974). No significant effects were seen in males at 9.6 mg/kg/day and in females at 19 mg/kg/day. The findings from Kociba et al. (1974) were used to derive a chronic-duration oral MRL of 0.1 mg/kg/day for 1,4-dioxane. An elevated incidence of hepatocytomegaly was observed in female rats treated with ≥350 mg 1,4-dioxane/kg/day (the lowest dose tested in females) and in males dosed with 530 mg/kg/day of the chemical in the drinking water for 2-years (NCI 1978); the NOAEL in males was 240 mg/kg/day. In another 2-year drinking water study in F344/DuCrj rats, liver hyperplasia, spongiosis, and cell foci developed in males dosed with ≥81 mg 1,4-dioxane/kg/day, but not with 16 mg/kg/day (JBRC 1998c). Females treated with 514 mg/kg/day also developed hyperplasia, spongiosis, and cell foci, but no such lesions were observed at 103 mg/kg/day.

Mice dosed with up to 860 mg 1,4-dioxane/kg/day via the drinking water for 90 weeks showed no treatment-related non-neoplastic liver lesions (NCI 1978). Although the investigators stated that hepatocytomegaly was commonly found in treated mice, the incidences were not significantly different than in controls and no trend was apparent. Crj:BDF_1 mice dosed with 251–323 mg 1,4-dioxane for 2 years had significantly increased serum transaminase activities, and no gross or histological alterations were reported in females treated with doses of up to 1,066 mg/kg/day of 1,4-dioxane (JBRC 1998c). However, male mice showed angiectasis (abnormal dilation of a blood vessel) in the liver at 768 mg/kg/day.

Renal Effects. Acute lethal doses of 1,4-dioxane in rodents caused kidney lesions ranging from kidney enlargement to extensive kidney degeneration (de Navasquez 1935; Kesten et al. 1939; Laug et al. 1939; Smyth et al. 1941). Severe kidney damage was seen also in an acute study in dogs (Schrenk and

Yant 1936). Hydropic changes of the proximal renal tubule were reported in male and female F344/DuCrj rats dosed with 2,960 and 2,750 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 2 weeks (JBRC 1998a); the corresponding NOAELs were 1,010 and 1,040 mg/kg/day. Treatment for 13 weeks resulted in nuclear enlargement of the proximal renal tubules at 760 and 870 mg 1,4-dioxane/kg/day in male and female rats, respectively (JBRC 1998b); higher doses also induced hydropic changes in the proximal tubules. The NOAELs for morphological alterations in the kidneys were 330 and 430 mg/kg/day in males and females, respectively. Other significant findings in this study were a decrease in urinary pH in males at ≥330 mg/kg/day and an increase in absolute and relative kidney weight in females at ≥200 mg/kg/day. In another study, rats dosed for up to 34 days with 1,428 mg 1,4-dioxane/kg/day, a dose that caused deaths, had vascular congestion in the kidneys and cell degeneration in the cortical epithelium (Fairley et al. 1934). Similar lesions were observed in mice treated in the same fashion with approximately 2,916 mg/kg for up to 67 days (Fairley et al. 1934). Changes resembling glomerulonephritis were reported in male Wistar rats exposed to approximately 584 mg 1,4-dioxane/kg/day in the drinking water for about 452 days (Argus et al. 1965). Treatment of female Crj:BDF_1 mice with 1,710 mg 1,4-dioxane/kg/day in the drinking water for 13 weeks increased absolute kidney weight and decreased urinary pH; urinary pH was decreased in males at 920 mg/kg/day. Doses of 580 and 920 mg/kg/day in males and females, respectively, did not cause any significant renal effects (JBRC 1998b).

Kociba et al. (1974) observed degenerative changes in the kidneys from Sherman rats that died after 2–4 months of drinking water that provided approximately 1,015 mg 1,4-dioxane to males and 1,599 mg/kg/day to females. At termination of this 2-year study, the kidneys of both males (≥94 mg/kg/day) and females (≥148 mg/kg/day) showed tubular epithelial degeneration and necrosis, and there was evidence of renal tubular regeneration as indicated by increased tubular epithelial regenerative activity. The NOAEL in males and females was 9.6 and 19 mg/kg/day, respectively (Kociba et al. 1974). In the NCI (1978) bioassay in Osborne-Mendel rats, kidney lesions consisting of vacuolar degeneration and/or focal tubular epithelial regeneration in the proximal cortical tubules and occasionally hyaline casts were seen with significantly higher incidence in treated males (≥240 mg/kg/day, dose-related) and in high-dose females (640 mg/kg/day). The pH of the urine was significantly decreased in male F344/DuCrj rats dosed with 398 mg 1,4-dioxane/kg/day in the drinking water for 2 years, but no change was observed at 81 mg/kg/day (JBRC 1998c). In females, treatment with 103 mg/kg/day resulted in the detection of blood in the urine, decreased urinary pH, and nuclear enlargement of the proximal tubules; the NOAEL for renal effects in females was 21 mg/kg/day.

No treatment-related kidney lesions were observed in $B6C3F_1$ mice treated via the drinking water with up to 860 mg 1,4-dioxane/kg/day for 90 weeks (NCI 1978). Similar treatment of male $Crj:BDF_1$ mice with 768 mg 1,4-dioxane/kg/day resulted in decreased pH of the urine and nuclear enlargement of the proximal tubule; no such changes were observed at 251 mg/kg/day (JBRC 1998c). In females, doses of 323 mg/kg/day induced the excretion of glucose and protein in the urine and hematuria, but no gross or histopathological alterations were reported with doses up to 1,066 mg/kg/day. The NOAEL for renal effects in females was 77 mg/kg/day.

Endocrine Effects. No gross or histological alterations were observed in endocrine (thyroid, parathyroid, adrenal, pituitary) and exocrine (pancreas, salivary) glands from rats and mice dosed with up to 2,020 and 2,700 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (JBRC 1998b), or in rats and mice dosed with up to 514 and 1,066 mg 1,4-dioxane/kg/day, respectively, for 2 years (JBRC 1998c). No gross or microscopic alterations were seen in the pituitary, adrenal, thyroid, and parathyroid glands from rats dosed with up to 1,599 mg 1,4-dioxane/kg/day in the drinking water for 2 years (Kociba et al. 1974; NCI 1978) or in the same organs from mice dosed in the same manner with up to 860 mg 1,4-dioxane/kg/day (NCI 1978). No further information was located on effects of 1,4-dioxane on endocrine parameters.

Dermal Effects. No gross or histological alterations were observed in the skin from rats and mice dosed with up to 2,020 and 2,700 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (JBRC 1998b), or in rats and mice dosed with up to 514 and 1,066 mg 1,4-dioxane/kg/day, respectively, for 2 years (JBRC 1998c). Treatment of rats with up to 640 mg 1,4-dioxane/kg/day in the drinking water for 2 years or mice with up to 860 mg 1,4-dioxane/kg/day had no significant effect on the gross or microscopic appearance of the skin (NCI 1978).

Ocular Effects. No gross or histological alterations were observed in the eyes from rats and mice dosed with up to 2,020 and 2,700 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (JBRC 1998b), or in rats and mice dosed with up to 514 and 1,066 mg 1,4-dioxane/kg/day, respectively, for 2 years (JBRC 1998c). No other relevant information was located.

Body Weight Effects. Administration of a single dose of 10 mg 1,4-dioxane/kg by gavage in water to rats reduced body weight gain by approximately 32% relative to controls in a 7-day period (Stott et al. 1981). According to the investigators, this was likely due to a reduction in food consumption, consistent with the histological observation that hepatocytes were depleted of glycogen. However, treatment with

10 mg/kg/day for 11 weeks had no significant effect on weight gain, and doses of 1,000 mg/kg/day for 11 weeks decreased body weight only about 9% relative to controls (Stott et al. 1981). In 2-week drinking water studies, doses of approximately 2,750–2,960 mg 1,4-dioxane/kg/day reduced body weight gain in F344/DuCrj rats (JBRC 1998a). In Crj:BDF_1 mice, a significant reduction in body weight gain occurred in males at 2,550 mg/kg/day, but not at 1,380 mg/kg/day (JBRC 1998a). Reduced body weight gain was also reported in female F344/DuCrj rats treated for 13 weeks with ≥870 mg 1,4-dioxane/kg/day and in male and female Crj:BDF_1 mice treated for the same duration with ≥1,830 mg/kg/day (JBRC 1998b). In the JBRC (1998a, 1998b) studies, reduction in weight gain was usually associated with reduced food consumption and/or reduced water consumption. Sherman rats treated with 1,015–1,599 mg 1,4-dioxane/kg/day for 2 years gained approximately 10% less weight throughout the study (estimated from graphic data in the paper) than controls or rats dosed with 94–148 mg/kg/day (Kociba et al. 1974). In the NCI (1978) bioassay, body weight of male rats in the high-dose group (530 mg/kg/day) and female mice (860 mg/kg/day) were lower than controls during the second year of the study. No data on food consumption were provided in these two chronic-duration studies. In another chronic study, treatment of male F344/DuCrj rats with up to 398 mg 1,4-dioxane/kg/day did not significantly affect body weight, but females dosed with 514 mg/kg/day gained 19% less weight than controls; the NOAEL in females was 103 mg/kg/day (JBRC 1998c). Neither food nor water consumption was significantly altered in this case. Male Crj:BDF_1 mice dosed with 768 mg 1,4-dioxane/kg/day for 2 years gained 43% less weight than controls, and females dosed with 323 and 1,066 mg/kg/day gained 15 and 45% less weight than controls, respectively (JBRC 1998c). The NOAELs for males and females were 251 and 77 mg/kg/day, respectively. In mice, the reductions in weight gain were accompanied by significant reductions in water consumption, but there were no significant changes in food consumption.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after oral exposure to 1,4-dioxane. No gross or histological alterations were observed in the lymph nodes, spleen, and thymus from rats and mice dosed with up to 2,020 and 2,700 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (JBRC 1998b), or in rats and mice dosed with up to 514 and 1,066 mg 1,4-dioxane/kg/day, respectively, for 2 years (JBRC 1998c). No histopathologic alterations were observed in the spleen, thymus, and lymph nodes from rats dosed via drinking water with up to 1,599 mg 1,4-dioxane/kg/day for 2 years or from mice dosed similarly with up to 860 mg/kg/day (Kociba et al.

1974; NCI 1978). These NOAEL values for lymphoreticular effects are listed in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to 1,4-dioxane. In an acute study in rabbits, a single gavage dose of 4,400 mg 1,4-dioxane/kg induced staggering in one of three rabbits and 6,600 mg/kg produced narcosis in one of three rabbits and was lethal to two other rabbits (Knoefel 1935). No further details were provided in this early study. Male and female F344/DuCrj rats dosed with 2,960 and 2,750 mg 1,4-dioxane/kg/day, respectively, for 2 weeks showed vacuolar changes in the brain (JBRC 1998a); the respective NOAELs were 1,010 and 1,040 mg/kg/day. Similar effects were reported in male and female F344/DuCrj rats dosed with 1,900 and 2,020 mg 1,4-dioxane/kg/day, respectively, for 13 weeks in the drinking water (JBRC 1998a); the respective NOAELs were 760 and 870 mg/kg/day. However, no significant alterations were seen in the spinal cord or sciatic nerve. In the same study, no histopathological alterations were observed in the brain, spinal cord, and sciatic nerve from Crj:BDF_1 mice dosed with up to 2,700 mg 1,4-dioxane/kg/day. No histopathologic alterations were observed in the brain, spinal cord, and sciatic nerve from rats dosed via the drinking water with up to 1,599 mg 1,4-dioxane/kg/day for 2 years or from mice dosed similarly with up to 860 mg/kg/day (JBRC 1998c; Kociba et al. 1974; NCI 1978). The NOAEL and LOAEL values for neurological effects are listed in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to 1,4-dioxane.

Standard reproductive toxicity studies on 1,4-dioxane were not located. Only ancillary information is available. No gross or histological alterations were observed in the reproductive organs (testes, prostate, seminal vesicles, epididymis, uterus, ovaries, vagina) from rats and mice dosed with up to 2,020 and 2,700 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (JBRC 1998b), or in rats and mice dosed with up to 514 and 1,066 mg 1,4-dioxane/kg/day, respectively, for 2 years (JBRC 1998c). No evidence of gross or microscopic alterations was found in the reproductive organs from rats exposed through the drinking water to up to 1,599 mg 1,4-dioxane/kg/day for up to 2 years (Kociba et al. 1974;

NCI 1978) or from mice exposed to up to 860 mg 1,4-dioxane/kg/day for up to 90 weeks (NCI 1978). These values are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to 1,4-dioxane. Only one relevant animal study was located. In that study, groups of pregnant Sprague-Dawley rats were administered 0, 258, 516, or 1033 mg 1,4-dioxane by gavage on gestation days 6–15 and sacrifices were conducted on gestation day 21 (Giavini et al.1985). Dams in the high-dose group gained less weight than controls and fetal weight in this group was reduced by 5.3% relative to controls. In addition, a slightly but significantly higher incidence of reduced sternum ossification was noticed in the high-dose group. No other significant differences between treated and control groups were observed, including number of implantations and of live fetuses, post-implantation loss, and incidence of malformations. The NOAEL and LOAEL values are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located regarding oral exposure of humans to 1,4-dioxane and cancer, but numerous studies have examined the carcinogenicity of 1,4-dioxane in animals exposed orally, in all of them the test material has been administered in the drinking water. In general, the studies in animals can be divided into a group in which numerous limitations are apparent, including small number of animals, low tumor incidences, lack of statistical analyses, and only one dose level was tested, and another small group of standard bioassays. To the former category belong Argus et al. (1965, 1973), Hoch-Ligeti and Argus (1970), and Hoch-Ligeti et al. (1970), whereas the standard bioassays include JBRC (1998c), Kociba et al. (1974), and NCI (1978).

Argus et al. (1965) exposed a group of 26 male Wistar rats to 1,4-dioxane in the drinking water at a concentration of 1% for 452 days. Nine rats served as controls. The maximal dose per rat was 132 g, which divided by an average exposure time of 452 days yields a daily dose of 584 mg/kg/day, assuming a reference body weight of 0.5 kg for mature male Wistar rats. End points examined included gross necropsy and histopathologic examination of tissues, but the range of tissues examined was not specified. Six of the 26 rats treated with 584 mg 1,4-dioxane/kg/day developed liver tumors that ranged in appearance from small neoplastic nodules to multifocal hepatocellular carcinomas. One treated rat had a

transitional cell carcinoma of the kidney and one rat that received a total dose of 116 g for 387 days (599 mg/kg/day) developed leukemia. One control rat developed a lymphosarcoma.

In a subsequent study by the same group of investigators, groups of male Sprague-Dawley rats (28–32/group) were treated with 1,4-dioxane in the drinking water for 13 months at levels of 0 (controls), 0.75, 1.0, 1.4, or 1.8% (Hoch-Ligeti et al. 1970). At termination, complete necropsy and histopathological examinations were conducted. Doses were estimated at about 400, 600, 800, and 1,000 mg/kg/day assuming that 13 months equals 390 days, a body weight of 0.6 kg for the rats, and the total dose provided in the study (104–256 g). Six treated rats developed tumors of the nasal cavity. All of the tumors consisted of squamous cell carcinomas with marked keratinization. The incidences were as follows: one at 0.75%, one at 1%, two at 1.4%, and two at 1.8%. Hepatocellular carcinomas were also observed in the rats that had nasal carcinomas in the 1.4 and 1.8% groups (Argus et al. 1973). In the latter study, in addition to incidences of hepatomas and hepatocellular carcinomas, the authors reported the incidences of "incipient" hepatomas. Two types of incipient hepatomas were observed, one consisting of large cells, apparently filled and distended with fat, and the other of finger-like strands of rather smaller hepatocytes with large hyperchromic nuclei and dense cytoplasm. According to Argus et al. (1973), these nodules showed all the histological characteristics of fully developed hepatomas. The following tumor incidences were reported: 4 incipient tumors at 0.75%, 9 incipient tumors at 1%, 13 incipient tumors and 3 hepatomas at 1.4%, and 11 incipient tumors and 12 hepatomas at 1.8% 1,4-dioxane. No tumors were found in the lungs. The authors stated that the effective tumor dose (TD5), the 50% tumor dose (TD50), and the maximum effective dose (TD95) were 72, 149, and 260 g, respectively, evaluated from the probit plot of the dose-response (Argus et al. 1973).

In the Kociba et al. (1974) study, groups of Sherman rats (60/sex/dose level) were treated with 1,4-dioxane in the drinking water at levels of 0 (controls), 0.01, 0.1, or 1% for 716 days. This corresponded to doses of 0, 9.6, 94, and 1,015 mg/kg/day in males and 0, 19, 148, and 1,599 mg/kg/day in females based on body weight and water consumption data. Treatment with 1,4-dioxane significantly increased mortality in high-dose males and females beginning at about 2–4 months of treatment. These rats showed degenerative changes in both the liver and kidneys. Body weight gain was significantly reduced in high-dose animals from the beginning of the study. Carcinogenic effects were limited to the liver and nasal turbinates. The investigators combined the incidence of tumors in males and females and expressed them as the effective incidences in the number of rats that were alive at 12 months. The incidence of all hepatic tumors was 2/106 (1.9%), 0/110 (0%), 1/106 (0.9%), and 12/66 (18.2%, p=00022) in controls, low-, mid-, and high-dose rats, respectively. The corresponding incidences of hepatocellular

carcinomas were 1/106 (0.9%), 0/110 (0%), 1/106 (0.9%), and 10/66 (15.2%, p=0.00033). Only three high-dose rats (one male and two females) had nasal carcinomas (p=0.05491) and were considered treatment-related by the investigators.

In the NCI (1978) bioassay, groups of Osborne-Mendel rats (35/sex/dose level) were administered 1,4-dioxane in the drinking water for 110 weeks. The estimated doses were 0 (controls), 240 and 530 mg/kg/day in males and 0, 350, and 640 mg/kg/day in males. Neoplasms associated with the administration of 1,4-dioxane occurred in the nasal cavity from males and females, liver from females, and testis/epididymis in males. The incidences of squamous cell carcinomas in the nasal turbinates were 0/33, 12/33 (36%), and 16/34 (47%) in control, low-, and high-dose males, respectively; the corresponding incidences in females were 0/34, 10/35 (29%), and 8/35 (23%). The first tumors were seen at week 52 in males and week 66 in females. Statistical analyses of these incidences revealed a significant dose-related trend and significant differences between treated groups and controls. The incidences of hepatocellular carcinomas in females were 0/31, 10/33 (30%), and 11/32 (34%) in controls, low-, and high-dose groups, respectively. A higher incidence of mesotheliomas of the vaginal tunics of the testis/epididymis was seen in treated males than in controls (2/33, 4/33, and 5/34 in controls, low-, and high-dose, respectively). The incidences of other neoplasms were not related to treatment with the test material by type, site, test group, or sex. Under the conditions of the study, 1,4-dioxane induced hepatocellular carcinomas in female rats and squamous cell carcinoma of the nasal turbinates in male and female rats.

In the JBRC (1998c) study, groups of Fischer 344/DuCrj rats (50/sex/dose level) received 1,4-dioxane in the drinking water at levels of 200, 1,000, and 5,000 ppm for 2 years (0, 16, 81, and 398 mg/kg/day for males; 0, 21, 103, and 514 mg/kg/day for females). Survival was significantly decreased in the high-dose groups due to liver and nasal tumors. Twenty-two of 50 high-dose male rats survived compared to 40/50 in controls; 24/50 of high-dose females survived compared to 38/50 in controls. In high-dose males (398 mg/kg/day), the incidence of nasal cavity tumors was 7/50 ($p<0.01$) compared to none in the other groups; in high-dose females (514 mg/kg/day), the incidence was 8/50 ($p<0.01$) compared to none in the other groups. The nasal tumors included squamous cell carcinomas, sarcomas, rhabdomyosarcoma, and esthesioneuroepithelioma. The incidence of combined hepatocellular adenoma or carcinoma in males was 0/50, 2/50, 4/49, and 33/50 ($p<0.01$) in the control, low-, mid-, and high-dose male rats; the corresponding incidences in females were 1/50, 0/50, 5/50, and 40/50 ($p<0.01$). High-dose males also had an increased incidence of mesothelioma of the peritoneum (28/50 compared to 2/50 in controls).

High-dose females had an increased incidence of mammary gland adenomas (16/50 compared to 6/50 in controls).

Two studies have been conducted in mice. Groups of B6C3F$_1$ mice (50/sex/dose level) were administered 1,4-dioxane in the drinking water for 90 weeks (NCI 1978). Based on body weight and water consumption data, the investigators estimated that the water provided doses of 0 (controls), 720, and 830 mg/kg/day in males, and 0, 380, and 860 mg/kg/day in females. Mortality was significantly increased (dose-related) in female mice. In female mice, 28/50, (56%) in the high-dose group, 39/50 (78%) in the mid-dose group, and 45/50 (90%) in the control group were still alive on week 91 of the study. In males, at least 90% of the mice in each group were still alive at week 91. Treatment with 1,4-dioxane significantly increased the incidence of liver tumors. The incidences of hepatocellular carcinoma were 2/49 (4%), 18/50 (36%), and 24/47 (51%), in controls, low-, and high-dose males, respectively; the corresponding incidences in females were 0/50, 12/48 (25%), and 29/37 (78%). The incidences of hepatocellular carcinomas or adenomas in males were 8/49 (16%), 19/50 (38%), and 28/47 (60%); the incidences in females were 0/50, 21/48 (44%), and 35/37 (95%). The incidences of these tumors were statistically significant for dose-related trend and for direct comparisons with controls. No other neoplasm, benign, or malignant, was found to be associated to treatment with 1,4-dioxane.

In another study (JBRC 1998c), groups of Crj:BDF$_1$ mice (50/sex/dose level) received 1,4-dioxane in the drinking water at levels of 500, 2,000, and 8,000 ppm for 2 years (0, 66, 251, and 768 mg/kg/day for males; 0, 77, 323, and 1,066 mg/kg/day for females). Early mortality occurred in female mice, and this was attributed to liver tumors. Survival rates at 104 weeks in females were 29/50, 29/50/ 17/50, and 5/50 in control, low-, mid-, and high-dose groups, respectively. A significant and dose-related increase in the incidence of liver adenomas and carcinomas of the liver was found in female mice. The incidences of combined adenomas and carcinomas in control, low-, mid-, and high-dose females were 4/50, 34/50, 41/40, and 46/50 ($p<0.01$ for all treated groups). High-dose males (768 mg/kg/day) also showed a significant increased incidence of hepatocellular carcinomas; the combined incidences of adenomas and carcinomas, as the dose increased, were 21/50 (controls), 31/50, 37/50, and 39/50 ($p<0.01$). There were no nasal cavity tumors in male or female mice.

In the single study in guinea pigs, a group of 22 male guinea pigs was administered 1,4-dioxane in the drinking water at concentrations of 0.5–2% for 23 months (Hoch-Ligeti and Argus 1970). Ten guinea pigs served as controls. The investigators stated that the total intake of 1,4-dioxane during the 23 months of the experiment was 588–625 g. Assuming a reference body weight of 0.84 kg and converting

23 months into 690 days (30 days/month), the intake of 1,4-dioxane was approximately 1,014–1075 mg/kg/day. All of the animals were sacrificed within 28 months. Very little additional data were presented in this brief note. Examination of the lungs revealed peri- or intrabronchial epithelial hyperplasia and nodular mononuclear infiltration in nine of the treated guinea pigs. In addition, two guinea pigs had carcinoma of the gall bladder, three had early hepatomas, and one had adenoma of the kidney. No tumors were found in the controls.

1,4-Dioxane was tested also as a cancer initiator in mice (Bull et al. 1986) and promoter in rats (Lundberg et al. 1987). Female Sencar mice were dosed with 1,000 mg 1,4-dioxane/kg by gavage before receiving topical applications of 1 μg of 12-O-tetradecanoylphorbol-13-acetate (TPA) 3 times/week for 20 weeks. A control group was initiated with acetone before the TPA application. Administration of 1,4-dioxane did not increase the formation of papillomas compared to mice initiated with acetone and promoted with TPA, indicating a lack of initiating activity under the conditions of the study. The tumor promotion activity of 1,4-dioxane was studied in groups of male Sprague-Dawley rats (8–11/group). All rats went through a 2/3 hepatectomy before receiving a single intraperitoneal injection of 30 mg/kg of diethylnitrosamine (DENA). Five days later, treatment with 100 or 1,000 mg/kg of 1,4-dioxane by gavage in saline started once daily, 5 days/week for 7 weeks. One week after the last treatment, the rats were killed, the liver was removed and stained for gamma-glutamyl-transpeptidase (GGT), and the number and total volume of GGT-positive foci was studied. 1,4-Dioxane alone had no significant effect on the end points evaluated. In DENA initiated rats, the high-dose of 1,4-dioxane induced a significant increase in the number of foci and total volume of foci relative to rats treated with DENA alone. The livers of high-dose rats showed enlarged foamy hepatocytes mainly in midzonal regions. The foamy appearance was due to vacuoles shown to contain fat. The results indicated promotion activity by 1,4-dioxane.

The data available indicate that 1,4-dioxane produced liver and nasal cancer in rats and liver tumors in mice. The EPA has derived an oral cancer potency factor of 0.011 $(mg/kg/day)^{-1}$ for 1,4-dioxane using the Linearized Multistage Model (IRIS 2004). This factor was calculated from oral exposure data reported by NCI (1978) regarding incidence of tumors of the nasal turbinates in male Osborne-Mendel rats. The lifetime average doses that would result in risk of $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, and $1x10^{-7}$ are $9x10^{-3}$, $9x10^{-4}$, $9x10^{-5}$, and $9x10^{-6}$ mg/kg/day, respectively, as indicated in Figure 3-2. The EPA is currently re-evaluating the health assessment for 1,4-dioxane (EPA 2004f).

3.2.3 Dermal Exposure

3.2.3.1 Death

As mentioned in Section 3.2.1.1, Johnstone (1959) described a fatal case of a worker exposed to 1,4-dioxane for only 1 week and whose post-mortem examination showed kidney and liver alterations. The room in which the patient had worked had no exhaust ventilation and the worker was not provided a respirator. Dermal exposure in this case may have been considerable since the worker used liquid 1,4-dioxane to keep his hands free of glue. A dermal LD_{50} of 7,600 mL/kg was reported for rabbits (RTECS 2004); this value is presented in Table 3-3.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, endocrine, or body weight effects in humans or in animals after dermal exposure to 1,4-dioxane. No studies were located regarding hepatic and renal effects in humans following dermal exposure to 1,4-dioxane.

The highest NOAEL and all reliable LOAEL values from each study for systemic effects in each species and duration category are recorded in Table 3-3.

Hepatic Effects. In a study with four guinea pigs, approximately 143 mg 1,4-dioxane/kg was applied to a clipped area of the nape 5 days/week for 49–101 days (Fairley et al. 1934). Upon sacrifice on days 49, 66, 77, and 101, no gross alterations of the liver were observed, but there were indications of patchy cell degeneration. The same protocol conducted in four rabbits applied doses of approximately 57 mg/kg showed vascular congestion of the liver and patchy cell degeneration in two of the rabbits (Fairley et al. 1934).

Renal Effects. Application of approximately 143 mg 1,4-dioxane/kg to a clipped area of the nape of guinea pigs 5 days/week for 49–101 days resulted in renal cortical cell degeneration and hemorrhages. The same experiment conducted in rabbits applied approximately 57 mg 1,4-dioxane/kg resulted in the same type of kidney lesions (Fairley et al. 1934).

Table 3-3 Levels of Significant Exposure to 1,4-Dioxane - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL				Reference Chemical Form
				Less Serious		Serious		
ACUTE EXPOSURE								
Death								
Rabbit (NS)	once					7600 ml/kg	(LD50)	RTECS 2004
Systemic								
Human	3 min	Ocular	2000 ppm					Fairley et al. 1934
Human	15 min	Ocular	200 B ppm	300 B ppm	(eye irritation)			Silverman et al. 1946
Human	10 min	Ocular		1600 ppm	(slight eye irritation)			Yant et al. 1930
Human	6 hr	Ocular		50 M ppm	(eye irritation)			Young et al. 1977
Rat (Wistar)	once	Dermal	8300 M mg/kg					Clark et al. 1984

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Table 3-3 Levels of Significant Exposure to 1,4-Dioxane - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL			Reference Chemical Form
				Less Serious		Serious	
INTERMEDIATE EXPOSURE							
Systemic							
Gn Pig (NS)	49-101 d 5 d/wk 2 x/d	Hepatic			143 mg/kg	(cloudy swelling and patchy cell degeneration)	Fairley et al. 1934
		Renal			143 mg/kg	(degeneration and necrosis of cortical tubules)	
		Dermal	143 mg/kg				
Rabbit (NS)	49-101 d 5 d/wk 2 x/d	Hepatic			57 mg/kg	(patchy cell degeneration)	Fairley et al. 1934
		Renal			57 mg/kg	(tubular cell degeneration)	
		Dermal	57 mg/kg				

d = day(s); Gn pig = guinea pig; hr - hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; ml/kg = milliliter per kilogram; mg/kg = milligram per kilogram; min = minute(s); NOAEL = no-observed-adverse-effect level; NS = not specified; ppm = parts per million; wk = week(s); x = time(s)

Dermal Effects. Application of a single dose of up to 8,300 mg 1,4-dioxane/kg to an uncovered area of the skin of rats produced no signs of skin irritation within the period of observation of 14 days (Clark et al. 1984). Application of approximately 143 mg 1,4-dioxane/kg 5 days/week for 40–101 days to a clipped area of the nape from guinea pigs did not produce skin irritation (Fairley et al. 1934). Similar results were obtained in rabbits applied approximately 57 mg 1,4-dioxane/kg using the same protocol (Fairley et al. 1934).

Ocular Effects. The ocular effects observed in humans and in animals described in Section 3.2.1.2 and listed in Table 3-1 are assumed to have occurred by direct contact of vapors of 1,4-dioxane with eyes and are also listed in Table 3-3.

No studies were located regarding the following health effects in humans or animals after dermal exposure to 1,4-dioxane:

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

The carcinogenicity and initiator and promoter properties of 1,4-dioxane have been evaluated. To test whether 1,4-dioxane is a complete carcinogen, 0.2 mL of a solution of 1,4-dioxane (unspecified concentration) were applied to the shaved back from Swiss-Webster mice for 60 weeks (King et al. 1973). Examination of the skin at week 60 revealed only one skin sarcoma and one lymphoma, suggesting that under the conditions of the study, 1,4-dioxane was not a complete carcinogen. King et al. (1973) also tested whether 1,4-dioxane is a promoter by applying 15 μg of dimethylbenzanthracene (DMBA) to groups of Swiss-Webster followed 1 week later by the application of 0.2 mL of a solution of 1,4-dioxane (unspecified concentration) to the shaved back for 60 weeks. At week 60, only 4 males and 5 females were still alive (out of 30/sex). Treatment with 1,4-dioxane in mice initiated with DMBA resulted in an increased number of tumors in the skin, lungs, and kidneys. The activity of 1,4-dioxane in promoting skin tumors was similar to that observed with croton oil as a promoter. However, croton oil led to a much higher multiplicity of skin tumors per mouse than 1,4-dioxane. Bull et al. (1986) tested 1,4-dioxane as an

initiator. In that study, female Sencar mice were applied topical doses of 1,000 mg 1,4-dioxane/kg before receiving topical applications of 1 μg 12-O-tetradecanoylphorbol-13-acetate (TPA) 3 times/week for 20 weeks. A control group received an application of acetone before the TPA application. 1,4-Dioxane did not increase the formation of papillomas compared to mice initiated with acetone and promoted with TPA.

3.3 GENOTOXICITY

Studies of the *in vitro* and *in vivo* genotoxicity of 1,4-dioxane are summarized in Tables 3-4 and 3-5, respectively. 1,4-Dioxane was not genotoxic in standard *in vitro* tests of gene mutation in bacteria in the presence or absence of metabolic activation (Haworth et al. 1983; Khudoley et al. 1987; Morita and Hayashi 1998; Nestmann et al. 1984; Stott et al. 1981). Kwan et al. (1990) tested 1,4-dioxane in a strain of *Photobacterium phosphoreum*, which is sensitive to chemicals that are DNA-damaging agents, DNA-intercalating agents, DNA-synthesis inhibitors, and direct mutagens. 1,4-Dioxane showed no activity in the absence of metabolic activation, but was not tested with metabolic activation. No evidence of DNA damage was seen in *Escherichia coli* K-12 *uvrB/recA* incubated with 1,4-dioxane with or without metabolic activation (Helmér and Bolcsfoldi 1992). A study in the yeast *Saccharomyces cerevisiae* strain D61M also gave negative results for chromosomal aneuploidy without activation (Zimmermann et al. 1985), but was not repeated in the presence of metabolic activation. Studies with isolated mammalian cells exposed to 1,4-dioxane have also yielded negative results. For example, assays for induction of micronuclei, sister chromatid exchanges, and chromosomal aberrations in Chinese hamster ovary cells (CHO) were negative with and without metabolic activation (Morita and Hayashi 1998). A similar study by Galloway et al. (1987) also found no increase in chromosomal aberrations in CHO cells but did observe a slight increase in the incidence of sister chromatid exchanges in the absence of activation. Morita and Hayashi (1998) and McGregor et al. (1991) found no increase in gene mutations in mouse lymphoma cells incubated with 1,4-dioxane. 1,4-Dioxane did not induce DNA damage in rat hepatocytes (Goldsworthy et al. 1991), but increased cell transformations in BALB/3t3 cells at cytotoxic concentrations (Sheu et al. 1988). A test for DNA single strand breaks in rat hepatocytes incubated with 1,4-dioxane yielded positive results only at cytotoxic concentrations of 1,4-dioxane (Sina et al. 1983).

Studies of *in vivo* exposure of organisms to 1,4-dioxane also have been mostly negative, although some positive results have been reported. The only information in humans is that no increases in chromosomal

Table 3-4. Genotoxicity of 1,4-Dioxane *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Salmonella typhimurium (TA100, TA98, TA1535, TA1537)	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Stott et al. 1981
S. typhimurium (TA100, TA1535)	Gene mutation	–	–	Nestmann et al. 1984
S. typhimurium (TA98, TA100, TA1530, TA1535, TA1537)	Gene mutation	–	–	Khudoley et al. 1987
Photobacterium phosphoreum	DNA damage	NT	–	Kwan et al. 1990
Escherichia coli K-12 *uvrB*/*recA*	DNA damage	–	–	Hellmer and Bolcsfoldi 1992
S. typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Morita and Hayashi 1998
E. coli (WP2, WP2 *uvrA*)	Gene mutation	–	–	Morita and Hayashi 1998
Saccharomyces cerevisiae (D61M)	Chromosomal malsegregation	NT	–	Zimmermann et al. 1985
Mouse lymphoma cells	Gene mutation	–	–	Morita and Hayashi 1998
CHO-K1 cells	Chromosomal aberrations	–	–	Morita and Hayashi 1998
CHO-K1 cells	Sister chromatid exchange	–	–	Morita and Hayashi 1998
CHO-K1 cells	Micronuclei	–	–	Morita and Hayashi 1998
Rat hepatocytes	DNA repair	–	–	Goldsworthy et al. 1991
CHO-W-B1 cells	Chromosomal aberrations	–	–	Galloway et al. 1987
CHO-W-B1 cells	Sister chromatid exchange	–	±	Galloway et al. 1987
Mouse lymphoma cells	Gene mutation	–	–	McGregor et al. 1991
BALB/3T3 cells	Cell transformation	NT	+	Sheu et al. 1988

– = negative result; + = positive result; ± = weak positive result; CHO = Chinese hamster ovary; NT = not tested

Table 3-5. Genotoxicity of 1,4-Dioxane *In Vivo*

Species (test system)	End point	Results	Reference
Human peripheral lymphocytes	Chromosomal aberrations	–	Thiess et al. 1976
Rat hepatocytes	DNA repair	–	Goldsworthy et al. 1991
Rat nasal epithelial cells	DNA repair	–	Goldsworthy et al. 1991
Mouse hepatocytes	Micronuclei	+	Morita and Hayashi 1998
Mouse peripheral blood	Micronuclei	–	Morita and Hayashi 1998
Rat hepatocytes	DNA alkylation or repair	–	Stott et al. 1981
Rat hepatocytes	DNA damage	+	Kitchin and Brown 1990, 1994
Mouse bone marrow	Micronuclei	–	Tinwell and Ashby 1994
Mouse bone marrow (C57BL6)	Micronuclei	+	Mirkova 1994
Mouse bone marrow (BALB/c)	Micronuclei	–	Mirkova 1994
Mouse bone marrow	Micronuclei	–	McFee et al. 1994
Drosophila (food)	Dominant lethal	–	Yoon et al. 1985
Drosophila (food)	Meiotic non-disjunction	+	Muñoz and Barnett 2002

– = negative result; + = positive result; ± = weak positive result

aberrations were observed in peripheral lymphocytes from a groups of six workers employed in 1,4-dioxane production relative to observations made in six control subjects (Thiess et al. 1976). Several studies have reported results regarding micronuclei formation. An assay in bone marrow cells from C57BL6 mice after single gavage doses of up to 3,600 mg 1,4-dioxane/kg found a dose-related increase in the incidence of micronuclei, but the results in BALB/c mice were negative (Mirkova 1994). A similar study by Tinwell and Ashby (1994) found that 1,4-dioxane did not induce micronuclei in bone marrow cells from CBA mice treated with a single oral dose of 1,800 mg/kg or from C57BL6 mice dosed with 3,600 mg/kg. Studies reported by McFee et al. (1994) of several trials conducted by two different laboratories yielded equivocal results for micronuclei formation in mouse bone marrow. More recent data by Morita and Hayashi (1998) in CD-1 mice treated with a single gavage dose of up to 3,000 mg 1,4-dioxane/kg showed an increase in micronuclei in hepatocytes, but not in peripheral blood reticulocytes. Hepatocytes from Sprague-Dawley rats dosed with a single dose of 1,000 mg 1,4-dioxane/kg by gavage showed no evidence of DNA alkylation or DNA repair activity (Stott et al. 1981). This dose level administered via the drinking water to the rats for 11 weeks induced minimal hepatocellular swelling, which was accompanied by increased DNA synthesis (Stott et al. 1981). In male Fischer 344 rats administered single doses of up to 2,000 mg 1,4-dioxane/kg by gavage, 1,4-dioxane did not induce replicative DNA synthesis in hepatocytes (Uno et al. 1994), but it did in a subsequent study by the same group of investigators (Miyagawa et al. 1999). In liver tissue from Sprague-Dawley rats given two doses of 2,550 or 4,200 mg 1,4-dioxane/kg, there was a dose-related increase in DNA damage (assessed by alkaline elution) and cytochrome P-450 content; no significant effect was seen at ≤840 mg/kg (Kitchin and Brown 1990). Administration of a single oral dose of 1,000 mg 1,4-dioxane/kg to Fischer 344 rats produced no evidence of hepatocyte DNA repair, and the same negative response was obtained in rats dosed for a week via drinking water containing up to 2% 1,4-dioxane (Goldsworthy et al. 1991). No DNA repair activity was also observed in nasal epithelial cells from rats given 1% 1,4-dioxane in the drinking water for 8 days followed by a single gavage dose of 1,000 mg/kg (Goldsworthy et al. 1991). 1,4-Dioxane did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* in one study (Yoon et al. 1985), but was positive for meiotic non-disjunction in another study in *D. melanogaster* (Muñoz and Barnett 2002).

Collectively, the information available suggests that 1,4-dioxane is a non-genotoxic compound, or at best, a weakly genotoxic compound.

3.4 TOXICOKINETICS

The absorption of 1,4-dioxane following oral or inhalation exposure is rapid and virtually complete. Absorption after dermal exposure appears to be much less extensive. 1,4-Dioxane is widely distributed and rapidly metabolized in both humans and animals either to β-hydroxyethoxyacetic acid (HEAA) or to its interconversion product, 1,4-dioxane-2-one, which can form under acid conditions. Both forms are rapidly and extensively eliminated in the urine. Metabolism to HEAA or 1,4-dioxane-2-one in rats is dose-dependent, becoming saturated at high doses. Unchanged 1,4-dioxane is excreted in the urine and the expired air, mainly at high dose levels. 1,4-Dioxane and its major metabolite show little tendency to accumulate in the body.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Young et al. (1977) exposed a group of four healthy male volunteers to 50 ppm of 1,4-dioxane vapor by inhalation for 6 hours. Plasma concentrations of 1,4-dioxane climbed rapidly during the first 2 hours of exposure, and more slowly thereafter, indicating an initial rapid absorption, followed by a plateau phase in which steady-state levels in plasma were being approached or had been reached. Based on the presence of 1,4-dioxane and its main metabolite (HEAA) in the urine, the calculated total absorbed dose of 1,4-dioxane was 5.4 mg/kg, and the calculated rate was 76.1 mg/hour.

In rats exposed by head-only inhalation to 50 ppm of 1,4-dioxane for 6 hours, a total absorbed dose of 71.9 mg/kg was estimated, based on the detection of the compound and its primary metabolite (HEAA) in the urine (Young et al. 1978a, 1978b). The concentration of 1,4-dioxane in the plasma was 7.3 µg/mL at the end of exposure. It is of note that the value for total absorbed dose in this study, on a per body weight basis, is considerably greater than that calculated from volunteers exposed to the same airborne concentration of 1,4-dioxane for the same length of time (Young et al. 1977).

3.4.1.2 Oral Exposure

Data on the absorption of 1,4-dioxane following oral exposure in humans are not available.

Young et al. (1978a, 1978b) exposed groups of male Sprague-Dawley rats to 10, 100, or 1,000 mg/kg of uniformly labeled ^{14}C-1,4-dioxane by gavage, and reported that <2% of the label was found in the feces in the first 24 hours (10 mg/kg dose) or 72 hours (100 or 1,000 mg/kg doses), indicating rapid and nearly-complete absorption of the compound from the gastrointestinal tract. In another experiment in the same manuscript (Young et al. 1978a, 1978b), groups of male Sprague-Dawley rats were exposed to 10, 100, or 1,000 mg/kg of uniformly labeled ^{14}C-1,4-dioxane by gavage daily for 17 days. Less than 2% of the total administered label was recovered in the feces in 480 hours post-exposure, indicating that at least 98% absorption had occurred.

3.4.1.3 Dermal Exposure

Data on the absorption of 1,4-dioxane in humans following dermal exposure are not available, but a lethal case of intoxication with 1,4-dioxane in which the patient had extensive dermal contact with 1,4-dioxane in addition to inhalation of vapors suggests that dermal absorption is possible (Johnstone 1959).

Marzulli et al. (1981) applied uniformly labeled ^{14}C-1,4-dioxane, dissolved in either methanol or skin lotion, to the unoccluded skin of Rhesus monkeys and measured the ability of the compound to penetrate the skin by analysis of radiolabel in the urine. The skin penetration of 1,4-dioxane was minimal, being <4% in all cases; however, because the skin was unoccluded, evaporation may have influenced the study results.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Data on the distribution of 1,4-dioxane following inhalation exposure in humans or animals are not available.

3.4.2.2 Oral Exposure

Data on the distribution of 1,4-dioxane following oral exposure in humans or animals are not available.

3.4.2.3 Dermal Exposure

Data on the distribution of 1,4-dioxane following dermal exposure in humans or animals are not available.

3.4.2.4 Other Routes of Exposure

The distribution of ^{3}H-1,4-dioxane was determined in male Sprague-Dawley rats after intraperitoneal injection of 6.97 mg/kg (Woo et al. 1977b). Levels of activity were measured in whole blood, liver, kidney, spleen, lung, colon, and skeletal muscle at 1, 2, 6, and 16 hours. The radioactivity was widely distributed, with concentrations of dioxane equivalents at 1 and 16 hours decreasing from 93.4 to 41.4 nmol/mL in the blood, from 59.1 to 24.2 nmol/g in the liver, from 116.1 to 31.9 nmol/g in the kidney, from 49.6 to 30 nmol/g in the spleen, from 52.2 to 23.2 nmol/g in the lung, from 56.1 to 27.7 nmol/g in the colon, and from 45.3 to 28.1 nmol/g in skeletal muscle. It should be noted that the tissue samples were not perfused or corrected for levels of blood in the tissue, so there might have been some influence of the blood-borne activity on the reported tissue values. Within the tissues, the percent covalent binding at 16 hours was universally <20%, with the highest levels in the colon (17.3% bound), spleen (16.4% bound), and liver (13.7% bound), followed by the lung (11.2% bound), kidney (9.5% bound), whole blood (3.1% bound), and skeletal muscle (2.7% bound). Within the cells, the highest activity levels were found in the cytosol (~68% at 6 hours post-exposure), with lesser amounts in the microsomal (~15% at 6 hours post-exposure), mitochondrial (~14% at 6 hours post-exposure), and nuclear (<3% at 6 hours post-exposure) fractions. Interestingly, percent covalent binding was entirely opposite in proportion to total activity levels, with the greatest percent binding found in the nuclear fraction (~65%), followed by the mitochondrial (~46%), microsomal (~34%), and cytosolic (~5%) fractions.

Mikheev et al. (1990) exposed rats to ^{14}C-1,4-dioxane by intraperitoneal injection, and evaluated the levels of radioactivity in the blood, brain, testes, liver, and kidney at 5, 15, and 30 minutes and at 1, 3, and 6 hours post-injection in order to determine the tissue:blood concentration ratios. For all evaluated tissues at all time points, the tissue:blood ratio was between 0.5 and 1.5, indicating that 1,4-dioxane is distributed evenly and does not appreciably accumulate in any of the evaluated tissues. The maximum accumulation time (Tmax) was 5 minutes for liver and kidney, and 15 minutes for the blood, brain, and testes.

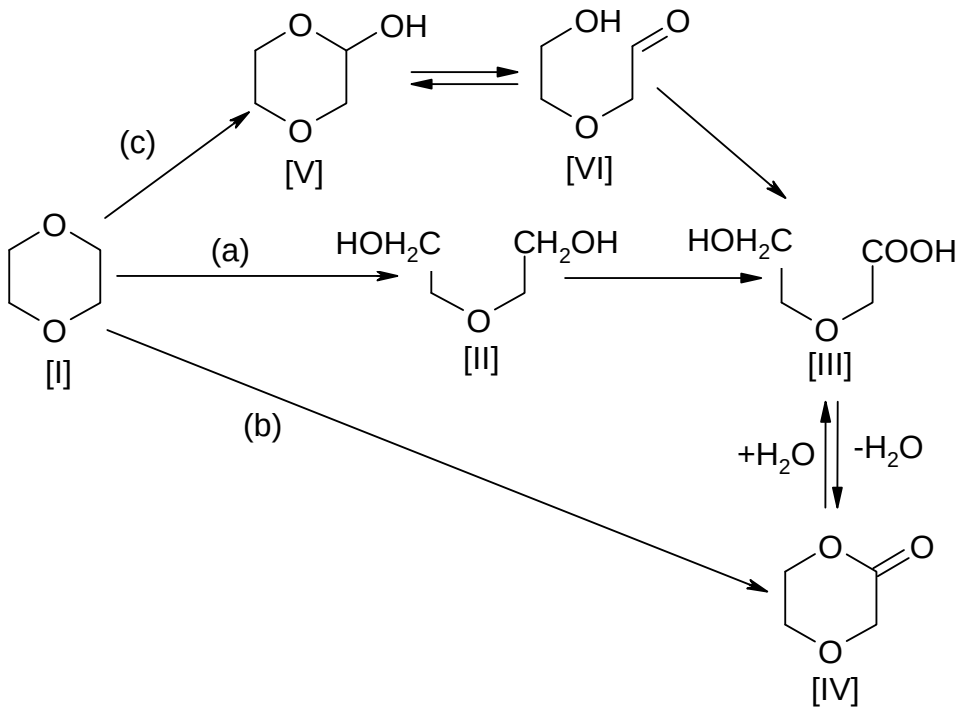
3.4.3 Metabolism

A proposed metabolism scheme for 1,4-dioxane is diagrammed in Figure 3-3.

The exact metabolic pathways of 1,4-dioxane metabolism are not known. However, numerous studies have reported that 1,4-dioxane is metabolized to a single urinary metabolite, believed to be HEAA. There is some question as to whether HEAA or 1,4-dioxane-2-one is the ultimate metabolite (Braun and Young 1977; Woo et al. 1977a, 1977b, 1977c; Young et al. 1977). This arises from the fact that under acid conditions, such as are often used in analytical assays, HEAA can be converted to 1,4-dioxane-2-one, and under alkaline conditions, the reverse reaction occurs. It is of note that HEAA is not volatile, and as a result, is often catalyzed to 1,4-dioxane-2-one in order to facilitate analysis, which may explain why Woo et al. (1977a, 1977d) reported 1,4-dioxane-2-one, rather than HEAA. As mentioned above, acid conditions, such as were employed by the assays of Woo et al. (1977a, 1977d) result in the formation of 1,4-dioxane-2-one from HEAA. Additional evidence for HEAA as the primary metabolite, rather than 1,4-dioxane-2-one, comes from a structure-activity relationship analyses of the genotoxicity of the two putative 1,4-dioxane metabolites (Blake 1995; Gombar 1995). 1,4-Dioxane-2-one is predicted to be strongly mutagenic, based on its structure, while HEAA would be only weakly genotoxic; the observed results of tests of genotoxicity for 1,4-dioxane correlate much closer with the predicted results from HEAA than from those of 1,4-dioxane-2-one. However, given the fact that the analytical methods used for analysis of the metabolites of 1,4-dioxane are capable of causing the formation of 1,4-dioxane-2-one from HEAA, and the reverse reaction as well, there is still some uncertainty as to the actual identity of the metabolite that has not been resolved.

Mixed-function oxidase enzymes, and cytochrome P-450 in particular, are critical to the metabolism of 1,4-dioxane, as induction of these enzymes increases the rate of HEAA formation, and inhibition decreases HEAA formation (Woo et al. 1977c, 1978). The initial step in metabolism is likely a P-450-catalyzed oxidative step; however the specific oxidation that occurs has not yet been determined.

Figure 3-3. Suggested Metabolic Pathways of 1,4-Dioxane in the Rat*



I = 1,4-dioxane; II = diethylene glycol; III = β-hydroxyethoxy acetic acid (HEAA); IV = 1,4-dioxane-2-one; V = 1,4-dioxane-2-ol; VI = β-hydroxyethoxy acetaldehyde

*Adapted from Woo et al. (1977c)

One possibility is diagrammed in pathway (a) of Figure 3-3. Cytochrome P-450 could act on one of the oxane oxygens, resulting in a decyclization and the formation of diethylene glycol. Evidence supporting this pathway comes from the fact that in animals injected with diethylene glycol, HEAA was found as the major metabolite (Woo et al. 1977a). Diethylene glycol could then be further metabolized to HEAA through an additional oxidative metabolic step. Alternately, cytochrome P-450 enzymes could act on one of the carbons in 1,4-dioxane to add a single oxygen atom, resulting in the direct formation of 1,4-dioxane-2-one as diagrammed in pathway (b) of Figure 3-3; however, no evidence is presently available to support this possible pathway. Another possibility is that rather than a single oxygen, a hydroxyl group could be added to a carbon atom, resulting in 1,4-dioxane-2-ol, as shown in pathway (c) of Figure 3-3. Additional oxidation to HEAA, resulting in a breaking of the ring structure, and further hydrolysis to HEAA could follow. As with pathway (b), there is no direct evidence supporting pathway (c) as the pathway for 1,4-dioxane metabolism.

1,4-Dioxane is extensively metabolized to HEAA in humans. Young et al. (1977) reported that over 99% of the urinary elimination of 1,4-dioxane after a 4-hour exposure of volunteers to 50 ppm occurred as HEAA rather than the parent compound. In a later study, the ratio of HEAA to dioxane in the urine of humans following a 7.5-hour exposure to 1.6 ppm dioxane was 118:1, indicating nearly complete metabolism at this exposure concentration.

The metabolism of 1,4-dioxane to HEAA in animals is nearly complete, as evidenced by studies examining the urine of exposed animals. Following inhalation exposure of rats to 50 ppm of 1,4-dioxane for 6 hours, the ratio of HEAA to dioxane in the urine over the 48-hour observation period was >3,000, indicating that for urinary elimination, nearly all of the compound was eliminated as the metabolite, rather than as the parent compound (Young et al. 1978a, 1978b).

The available animal data indicate that the metabolism of 1,4-dioxane is saturable. Young et al. (1978a, 1978b) reported that with an increasing oral dose level, a greater percentage of the total dose was eliminated as expired 1,4-dioxane, suggesting that the normally-rapid metabolism of 1,4-dioxane had reached a maximum, allowing the free compound to circulate in the blood and be eliminated by respiration; no dose-related differences were seen in elimination as CO_2 or in the feces that could otherwise account for this difference. A similar pattern was seen following 17 repeated doses of 10 or 1,000 mg/kg of ^{14}C-1,4-dioxane, with a greater elimination of label, primarily as the metabolite, in the urine at the lower dose, with the higher dose resulting in a greater elimination, as both ^{14}C-1,4-dioxane

and $^{14}CO_2$, in the expired air (Young et al. 1978a, 1978b). In an intravenous study reported in the same manuscript, the metabolic clearance of 1,4-dioxane decreased from 2.82 mL/minute following a single injection of 10 mg/kg to 0.17 mL/minute following an injection of 1,000 mg/kg, indicating that the metabolic capacity to metabolize 1,4-dioxane to HEAA had been saturated.

Woo et al. (1977a) reported that in the urine of rats orally exposed to 1–4 g/kg, only one metabolite was detected by gas chromatography. This metabolite was identified as 1,4-dioxane-2-one using nuclear magnetic resonance (NMR), infrared, and gas chromatograph-mass spectroscopy. Administration of diethylene glycol to rats resulted in the formation of the same metabolite, leading the study authors to hypothesize that diethylene glycol may represent an intermediate metabolite in the formation of 1,4-dioxane-2-one. In a later study by the same authors (Woo et al. 1977b), urine samples were collected, with glacial acetic acid as a preservative, from rats for 2 days following intraperitoneal injection of 50–400 mg/kg 1,4-dioxane. Gas chromatography identified a single metabolite, which was confirmed to be 1,4-dioxane-2-one by NMR, infrared, and mass spectroscopy.

Braun and Young et al. (1977) exposed groups of rats to radiolabeled 1,4-dioxane and characterized the major radiolabeled metabolite in the urine. The metabolite behaved identically to standards of both HEAA and 1,4-dioxane-2-one when evaluated using gas chromatography coupled with mass spectroscopy, preventing determination of the identity of the metabolite by this method. Using thin-layer chromatography, the metabolite's R_f value (the ratio of spot distance traveled to distance of the solvent front) of 0.60 correlated with that of HEAA (0.61) rather than that of 1,4-dioxane-2-one (1.00). The study authors therefore concluded that the identity of the urinary metabolite in rats was HEAA, rather than 1,4-dioxane-2-one, but noted the tendency for HEAA to cyclize under acidic conditions, forming 1,4-dioxane-2-one.

Woo et al. (1977c, 1978) pretreated groups of rats with phenobarbital (PB), Arochlor 1254 mixture (PCB), or 3-methylcholanthrene (MC) and examined the effects on the metabolism of an intraperitoneal dose of 1,4-dioxane. Pretreatment with PB resulted in a much more rapid metabolism of 1,4-dioxane, with the majority of the dose eliminated in the urine as HEAA within 32 hours, compared to 40 hours for the controls. The addition of 2,4-dichloro-6-phenylphenoxy ethylamine (DPEA), a cytochrome P-450 inhibitor, resulted in a reversal of this effect. Pretreatment with PCB resulted in similar effects as PB, while pretreatment with MC had no effect. Pretreatment with cobaltous chloride, to suppress P-450 synthesis, resulted in a decreased metabolite elimination, further implicating cytochrome P-450 enzymes in the metabolism of 1,4-dioxane.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

1,4-Dioxane and its metabolite, HEAA, were found in the urine of workers exposed to a time-weighted average concentration of 1.6 ppm of 1,4-dioxane for 7.5 hours (Young et al. 1976). The concentration of HEAA was 414 µmol/L, and that of unchanged 1,4-dioxane was only 3.5 µmol/L suggesting rapid and extensive metabolism. In four volunteers exposed to 50 ppm of 1,4-dioxane for 6 hours, over 99% of the urinary elimination of the compound occurred as its metabolite HEAA (Young et al. 1977) during the exposure period or within the 18 hours post-exposure; the remainder of the urinary elimination occurred as the parent compound. The half-life of elimination of 1,4-dioxane from plasma was 59 minutes, of dioxane in urine was 48 minutes, and of HEAA in the urine was 2.7 hours. The urinary elimination data suggested that elimination kinetics of 1,4-dioxane and HEAA are best described with first-order, one-compartment kinetic models. Elimination by other pathways (e.g., feces, expired air) was not evaluated in this study.

Following inhalation exposure in animals, the primary route of elimination is believed to be the urine. Young et al. (1978a, 1978b) reported that following inhalation exposure in rats, urinary elimination of 1,4-dioxane was primarily as HEAA, rather than as the parent compound.

3.4.4.2 Oral Exposure

Data on the elimination of 1,4-dioxane in humans following oral exposure are not available.

The administered dose of 1,4-dioxane has an effect on elimination of the compound. While urinary elimination is the predominant pathway regardless of dose, at large doses, elimination in the expired air plays a greater role, possibly due to the saturable pathways of 1,4-dioxane metabolism. After single oral doses of ^{14}C-1,4-dioxane in rats, 99% of the label was recovered in the urine and <1% was recovered in the expired air at 10 mg/kg; 86% of the label was recovered in the urine and 4.7% in the expired air at 100 mg/kg; and 76% of the label was found in the urine and 25% in the expired air at 1,000 mg/kg (Young et al. 1978a, 1978b). Similar results were seen following 17 daily gavage doses of ^{14}C-1,4-dioxane in rats, with 99 and 83% of the label found in the expired air, 1.3 and 8.9% of the label found as expired dioxane, and 4.1 and 7% found as expired CO_2 in animals receiving 10 and 1,000 mg/kg,

respectively. Elimination of 1,4-dioxane in both the expired air and in the urine appear to be first-order kinetic processes (Young et al. 1978a, 1978b).

3.4.4.3 Dermal Exposure

Data on the elimination of 1,4-dioxane following dermal exposure in humans and animals are not available.

3.4.4.4 Other Routes of Exposure

After a single intravenous dose of 10 mg/kg of 1,4-dioxane in rats, 4% of the dioxane was eliminated in the urine as dioxane, 92% as HEAA, and 1% was eliminated in the expired air (Young et al. 1978a, 1978b). Following a 1000 mg/kg dose, 11% was eliminated in the urine as dioxane, 60% as HEAA, and 27% in the expired air, indicating a dose-related shift in the elimination of the compound, possibly due to metabolic saturation. At low intravenous doses, 1,4-dioxane is eliminated from the plasma with apparently linear kinetics, while higher doses are eliminated progressively more slowly, achieving linear kinetics only after a non-linear phase, indicating the involvement of a saturable process, very likely metabolic saturation, in elimination of the compound. Elimination of 1,4-dioxane in both the expired air and in the urine following intravenous exposure appear to be first-order kinetic processes (Young et al. 1978a, 1978b). Pretreatment of rats with phenobarbital resulted in a 2.7-fold greater elimination of HEAA in the urine than in rats that were not pretreated (Woo et al. 1977c, 1978). The addition of DPEA partly reduced this effect, with the PB + DPEA animals eliminating 1.8-fold the HEAA of controls.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based

pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste

sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

Leung and Paustenbach (1990) Model.

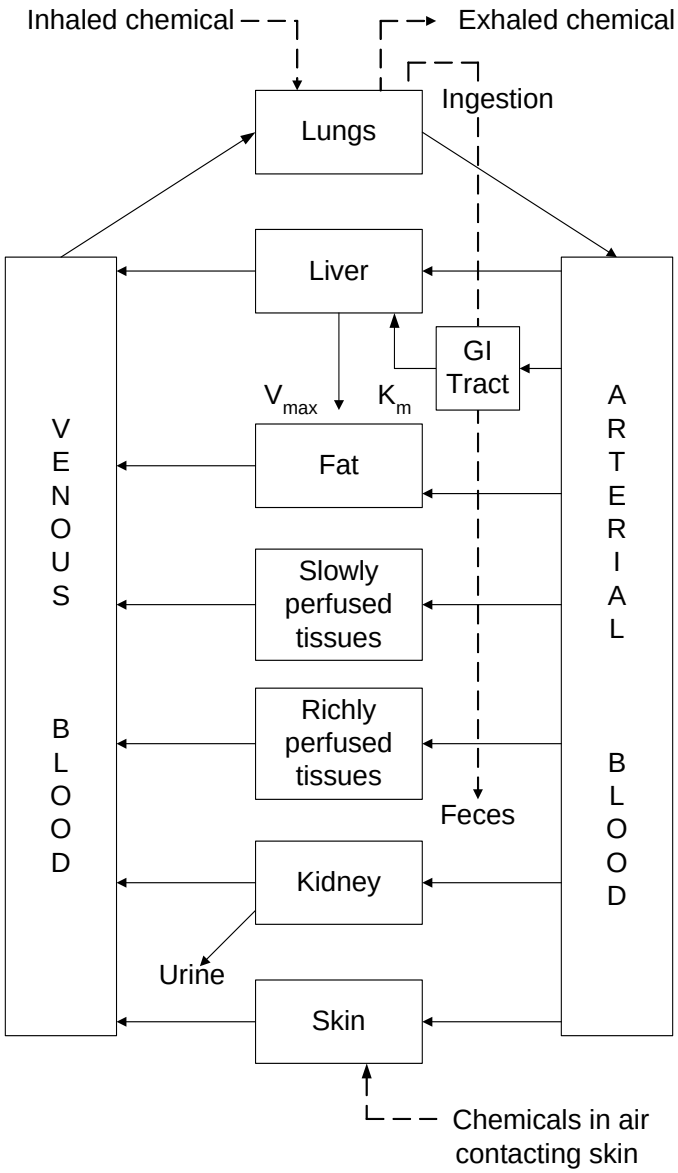
Leung and Paustenbach (1990) developed a PBPK model for 1,4-dioxane for rats and humans, based on the styrene model of Ramsey and Andersen (1984). The model simulates concentrations of 1,4-dioxane in the four modeled tissue compartments, as well as in arterial and venous blood, and the amount of metabolites formed.

Description of the Model. The model consists of compartments for liver, fat, slowly perfused tissues, and richly perfused tissues. Model parameters are presented in Table 3-6. Model inputs included inhalation, where 1,4-dioxane input was assumed to occur at a rate equal to the cardiac output, and drinking water, where 1,4-dioxane absorption was considered to be a zero-order process depositing 1,4-dioxane directly into the liver compartment. Tissue/air partition coefficients for rat blood, liver, fat, and muscle were determined by vial equilibration, and tissue/blood values were calculated by dividing the tissue/air coefficient by the blood/air coefficient. The richly perfused coefficient was set equal to that of the liver. Human coefficients were assumed to be identical to the rat. For the human model, alveolar ventilation rate and cardiac output were estimated using a $(\text{body weight})^{0.74}$ scalar. The metabolic constants were obtained by optimization of the model with experimental data from Young et al. (1977, 1978a, 1978b); these studies were also used to calibrate the model, using data on blood 1,4-dioxane levels, 1,4-dioxane in expired air, and urinary excretion of 1,4-dioxane and HEAA to compare with model predictions.

Validation of the Model. The model parameters were optimized against the rat and human data of Young et al. (1977, 1978a, 1978b). Comparisons of model simulations against data from studies other than those used in model development were not presented.

Risk Assessment. The model attempts to estimate concentrations of 1,4-dioxane in the blood and in the tissue compartments, as well as the levels of metabolites formed, following an inhalation or oral exposure to 1,4-dioxane. These internal dose surrogates could be used in the assessment of health risks from exposure to 1,4-dioxane. The study authors used liver tumor data from a rat study (Kociba et al.

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Table 3-6. Parameters Used in the PBPK Model for 1,4-Dioxane

	Rat	Human
Weights		
Body (kg)	0.25	84.1
Liver (percent)	4	4
Fat (percent)	7	20
Richly perfused (percent)	5	5
Slowly perfused (percent)	75	62
Blood Flow		
Cardiac output (L/hour)	5.4	399
Liver (percent)	25	25
Fat (percent)	5	5
Richly perfused (percent)	51	51
Slowly perfused (percent)	19	19
Air flow		
Alveolar ventilation (L/hour)	5.4	399
Partition coefficients		
Liver/blood	0.85	0.85
Fat/blood	0.4	0.4
Richly perfused/blood	0.85	0.85
Slowly perfused/blood	0.54	0.2[a]
Metabolic constants		
Vmax	1.9[a]	300[a]
Km	7.5[a]	15[a]

[a]Values obtained by model optimization

Source: Adapted from Leung and Paustenbach (1990)

1974) to estimate risk-specific doses for tumor formation following oral exposure, fitting the rat data to a multistage model and using liver dioxane concentrations as the dose metric for conversion from rat to human values. Human risk levels calculated by dividing the rat value by 5.5 (a body surface area scaling factor) were compared with values calculated using the model to calculate a human equivalent concentration; the model values resulted in a 7–8-fold greater value for maximum likelihood exposure (MLE) risk values than did division of the rat values by 5.5. It is important to note that the application of a PBPK model only addresses differences in pharmacokinetic behavior between species, and that differences in pharmacodynamic behavior must be discussed separately.

Target Tissues. The model simulates concentrations of 1,4-dioxane in the four modeled tissue compartments, as well as in arterial and venous blood, and the amount of metabolites formed. While two of the compartments represent actual body tissues (liver, fat), it is not known whether the model's estimates of tissue concentrations of 1,4-dioxane in these tissues is representative of the actual concentration in the tissues. However, the model's predictions of metabolite formation have been calibrated with actual data, providing evidence that the estimate of internal dose to the liver (where all metabolism is assumed to occur) is accurate.

Species Extrapolation. The Leung and Paustenbach (1990) PBPK model for 1,4-dioxane was developed in rats and humans, and human data on the pharmacokinetics of 1,4-dioxane was used in the optimization of model parameters. As such, interspecies extrapolation using the two models should be possible, although it has not yet been presented.

Interroute Extrapolation. The model includes inputs for both inhalation and oral exposures, and as such, should provide a means to estimate an internal dose to a target tissue compartment or other dose metric regardless of which of these two exposure routes is used. The use of the model for interroute extrapolation is therefore feasible, although it has not yet been performed.

Reitz et al. (1990) Model.

Reitz et al. (1990) have also published a PBPK model for 1,4-dioxane in rats, mice, and humans, again building on the basic structure of the Ramsey and Andersen (1984) model for styrene. The model estimates concentrations of 1,4-dioxane in the modeled tissue compartments, as well as in arterial and venous blood, and the amount of metabolites formed.

Description of the Model. The model consisted of six compartments: lung, fat, liver, venous blood, slowly perfused tissues, and richly perfused tissues. The model was designed to simulate inhalation, intravenous, and oral exposures. Oral exposures could be by gavage or in the drinking water, and were assumed to pass through the liver before entering the systemic circulation. Intravenous injection was simulated by direct addition to the venous blood compartment, while inhalation deposited directly into arterial blood at a rate dependent on ventilation, cardiac output, and the blood/air partition coefficient for 1,4-dioxane. Tissue/air partition coefficients for 1,4-dioxane in human blood, rat blood, rat fat, rat muscle, and rat liver were determined by vial equilibration. Organ volumes, blood flows, and air flows were similar to those employed by Andersen et al. (1987), except that ventilation and cardiac output rates in humans were increased to provide a better simulation of the human blood level data. Metabolic rate constants were determined from data presented by Young et al. (1977, 1978a, 1978b) during optimization of the model. Metabolism was assumed to occur only in the liver, and metabolites were assumed to be removed from the system. Elimination of parent compound was modeled in the expired air and in the urine. The model parameters are presented in Table 3-7. After optimization using data from Young et al. (1977, 1978a, 1978b) the results of model runs and the corresponding experimental data were presented for venous blood concentrations in rats and humans following inhalation exposure, and venous blood concentrations in rats following intravenous exposure.

Validation of the Model. The model parameters were optimized against the rat and human data of Young et al. (1977, 1978a, 1978b). Comparisons of model simulations against data from studies other than those used in model development were not presented.

Risk Assessment. The model attempts to estimate concentrations of 1,4-dioxane in the blood and in the tissue compartments, as well as the levels of metabolites formed, following an inhalation, oral, or intravenous exposure to 1,4-dioxane. These internal dose surrogates could be used in the assessment of health risks from exposure to 1,4-dioxane. The study authors used the model to estimate “Human Virtually Safe Doses” (VSDs) based on tumor data from oral and inhalation studies in rats and mice (Kociba et al. 1974; NCI 1978; Torkelson et al. 1974). The VSDs were calculated by converting the rat no observable effect level (NOEL) for tumor formation to a human equivalent dose, and then dividing by a safety factor 100. The authors calculated a risk-specific water concentration of 20,000 µg/L for upper bound lifetime cancer risk of 1 in 100,000, calculated to represent the lower 95% confidence limit on administered dose producing a lifetime increase in risk of developing liver cancer, using the weighted average of area under the liver concentration/time curve and area under the metabolite concentration/time

Table 3-7. Parameters Used in the Reitz et al. (1990) PBPK Model for 1,4-Dioxane

	Mice	Rats	Humans
Body weight (kg)	0.035	0.400	70.0
Percentage of body weight			
Liver	4.0	4.0	3.1
Fat	4.0	7.0	23.1
Rapidly perfused	5.0	5.0	3.7
Slowly perfused	73.0	70.0	56.1
Blood	5.0	5.0	5.0
Flows (L/hour)			
Alveolar ventilation	2.34	7.61	696
Cardiac output	2.34	7.61	696
Percent of cardiac output			
Liver	25.0	25.0	25.0
Fat	5.0	5.0	5.0
Rapidly perfused	52.0	52.0	52.0
Slowly perfused	18.0	18.0	18.0
Partition coefficients			
Blood/air	2,750	1,850	3,650
Liver/air	1,557	1,557	1,557
Fat/air	851	851	851
Rapidly perfused/air	1,557	1,557	1,557
Slowly perfused/air	1,557	1,557	1,557
Saline/air	2,066	2,066	2,066
Metabolic constants (allometric)			
VmaxC (mg/hour)	10.0	13.7	6.35
Km (mg/L)	16.2	29.4	3.00
Miscellaneous constants			
Ka ($hour^{-1}$)	5.0	5.0	5.0
K_{ME} ($hour^{-1}$)	0.42	0.28	0.56
Water consumption (mL/day)	9.8	54	2,000

curve as the dose surrogate for conversion between species. It is important to note, however, that the application of a PBPK model only addresses differences in pharmacokinetic behavior between species, and that differences in pharmacodynamic behavior must be discussed separately.

Target Tissues. The model simulates concentrations of 1,4-dioxane in the four modeled tissue compartments, as well as in arterial and venous blood, and the amount of metabolites formed. While three of the compartments represent actual body tissues (liver, fat, venous blood), only for venous blood levels have experimental data been compared to model simulations. It is not known whether the model's estimates of tissue concentrations of 1,4-dioxane in the other tissues is representative of the actual concentration in the tissues. However, the model's predictions of metabolite formation have been calibrated with actual data, providing evidence that the estimate of internal dose to the liver (where all metabolism is assumed to occur) is accurate.

Species Extrapolation. The Reitz et al. (1990) PBPK model for 1,4-dioxane was developed for rats, mice, and humans. Human and rat data on the pharmacokinetics of 1,4-dioxane were used in the optimization of model parameters; mouse parameters were generally assumed to be equivalent to those in the rat. As such, interspecies extrapolation using the models for the different species should be possible.

Interroute Extrapolation. The model includes inputs for both inhalation, oral, and intravenous exposures, and as such, should be able to estimate an internal dose to a target tissue compartment or other dose metric regardless of which of these exposure routes is used. The use of the model for interroute extrapolation is therefore feasible, although it has not yet been performed.

Fisher et al. (1997) Model.

Fisher et al. (1997) have published a general PBPK model for volatile organic chemicals, which incorporates a compartment for elimination of the chemical in the breast milk. Model simulations predicted a high degree (18%) of lactational transfer of 1,4-dioxane. While the study authors note that the model is applicable to 1,4-dioxane, simulations using the model have not been compared to data from humans or animals exposed to 1,4-dioxane.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. The absorption of 1,4-dioxane following inhalation or oral exposure is rapid and essentially complete; absorption following dermal exposure is very low. The mechanisms involved in the absorption of 1,4-dioxane have not been evaluated, but given the speed of the absorption and the chemical similarity of 1,4-dioxane to other low-molecular-weight compounds, absorption is generally assumed to occur through passive diffusion.

Distribution. The mechanisms of distribution of 1,4-dioxane have not been evaluated. Data on the distribution of 1,4-dioxane are limited to studies following injection of the compound. In those studies, distribution of 1,4-dioxane was rapid (5–15 minutes to Tmax). 1,4-Dioxane has been detected in all tissues that have been evaluated, but has not been shown to appreciably accumulate in tissues, possibly due to its high water solubility.

Metabolism. Studies on the metabolism of 1,4-dioxane have clearly identified the primary metabolite as HEAA/1,4-dioxane-2-one, but have not confirmed a clear pathway for the formation of metabolites from 1,4-dioxane. Cytochrome P-450 enzymes are clearly involved, as evidenced by the studies of Woo et al. (1977c, 1978). It has been suggested that P-450-mediated metabolism may result in the formation of diethylene glycol, since injection of diethylene glycol in rats also results in the formation of HEAA (Woo et al. 1977a); however, additional data supporting this pathway have not been presented.

Excretion. The elimination of 1,4-dioxane occurs primarily (>95%) in the urine, as the primary metabolite, at low doses. At higher doses, metabolism becomes saturated, and a greater fraction is eliminated in the expired air; however, urinary elimination remains the primary method of elimination. Elimination of 1,4-dioxane in both the expired air and the urine following intravenous exposure appear to be first-order kinetic processes (Young et al. 1978a, 1978b). Evidence for active secretion or uptake of 1,4-dioxane from the kidney has not been reported.

3.5.2 Mechanisms of Toxicity

The mechanism of carcinogenicity of 1,4-dioxane has not been elucidated, but the results from several lines of investigation suggest that 1,4-dioxane has a non-genotoxic mode of action (Goldsworthy et al.

1991; Leung and Paustenbach 1990; Stott et al. 1981). Briefly, the collective evidence from evaluations of genetic toxicity suggests that 1,4-dioxane is unlikely to be genotoxic (see Section 3.3, Genotoxicity). 1,4-Dioxane was not mutagenic in bacterial assays with or without metabolic activation (Haworth et al. 1983; Khudoley et al. 1987; Morita and Hayashi 1998; Nestmann et al. 1984; Stott et al. 1981), did not induce chromosomal aneuploidy in yeast (Zimmermann et al. 1985), mutations in mouse lymphoma cells (Morita and Hayashi 1998; McGregor et al. 1991), or sex-linked recessive lethal mutations in *D. melanogaster* (Yoon et al. 1995). Moreover, in an occupationally study there was no evidence of an increased incidence of chromosomal aberrations among workers chronically exposed to relatively low levels of 1,4-dioxane compared to controls (Thiess et al. 1976). No significant increase in chromosomal aberrations was observed in Chinese hamster ovary cells incubated with 1,4-dioxane with or without metabolic activation, but there was a weak increase in sister chromatid exchanges when the cells were incubated without metabolic activation (Galloway et al. 1987). Also, incubation of BALB/3t3 cells with 1,4-dioxane resulted in transformations leading to the formation of foci, but at concentrations of 1,4-dioxane that were cytotoxic to over 50% of the cells (Sheu et al. 1998).

Several, structure-activity analyses have been conducted to study the mechanism of carcinogenicity of 1,4-dioxane. For example, a computerized structure relationship analysis using TOPKAT (version 3.0) in male rat and female mouse models showed a positive influence for the -O-CH_2- fragment of the molecule in both models, although the male rat model indicated that the symmetry of the 1,4-dioxane molecule plays a more substantial role in the carcinogenicity of 1,4-dioxane than the -O-CH_2- fragment (Blake 1995). An additional analysis of the potential role of 1,4-dioxane's metabolites HEAA and 1,4-dioxane-2-one predicted HEAA to be non-carcinogenic and non-mutagenic, but 1,4-dioxane-2-one was predicted to be strongly positive in the female mouse carcinogenicity model and the Ames mutagenicity model (Gombar 1995). A structure-activity relationship analysis using the Computer-Automated Structure Evaluation (CASE) methodology predicted that 1,4-dioxane would induce micronuclei in mice bone marrow cells as a result of the -O-CH_2- fragment. According to unpublished results cited by Rosenkranz and Klopman (1992), the concordance between experimental results and CASE predictions of the induction of micronuclei is in excess of 83%. However, Rosenkranz and Klopman (1992) indicated that the -O-CH_2- fragment does not seem to have intrinsic electrophilicity and could not suggest a possible DNA-reactive metabolite. This led them to suggest that the -O-CH_2- fragment might contribute to a non-genotoxic effect of 1,4-dioxane resulting in the induction of micronuclei. Since the experimental results of micronuclei-induction studies in mice have been mixed (McFee et al. 1994; Mirkova 1994; Morita and Hayashi 1998; Tinwell and Ashby 1994), Ashby (1994) suggested that it is not always possible to categorize a chemical as either unequivocally positive or negative in genotoxicity activity.

Numerous studies have examined the possible role of DNA damage, DNA synthesis, cell proliferation, or peroxisome proliferation in the carcinogenic effects of 1,4-dioxane. For instance, a test for DNA single strand breaks in rat hepatocytes incubated with 1,4-dioxane gave positive results, although only at cytotoxic concentrations (Sina et al. 1983). Stott et al. (1981) reported that hepatocytes from Sprague-Dawley rats dosed with 1,4-dioxane for 11 weeks showed no evidence of DNA alkylation or DNA repair activity, but showed increased levels of DNA synthesis. Based on these results, Stott et al. (1981) suggested that 1,4-dioxane induces tumors by a non-genetic mechanism.

The role of cell proliferation in the carcinogenicity of 1,4-dioxane was further evaluated in two studies that yielded inconclusive results. Administration of single doses of up to 2,000 mg 1,4-dioxane/kg by gavage to male Fischer 344 failed to induce replicative DNA synthesis in hepatocyte (Uno et al. 1994), which led the authors to suggest that 1,4-dioxane may induce liver cancer only in initiated cells. However, in a subsequent study by the same group of investigators, and using the same experimental protocol, 1,4-dioxane did increase hepatocyte cell proliferation (Miyagawa et al. 1999); the reason for the discrepancy in the results between the two studies is not apparent.

In liver tissue from Sprague-Dawley rats given single doses of 1,4-dioxane, there was a small but statistically significant amount of DNA damage (assessed by alkaline elution) at dose levels that did not induce cytotoxicity (Kitchin and Brown 1990, 1994). Liver toxicity was assessed by light microscopy and measurements of serum levels of ALT (no significant increase was observed). The DNA damage was accompanied by an increase in cytochrome P-450 content and by large increases in the activity of hepatic ornithine decarboxylase, suggesting a strong promoter activity for 1,4-dioxane.

Another study of the potential mechanisms of carcinogenicity of 1,4-dioxane showed that neither 1,4-dioxane nor the metabolite 1,4-dioxane-2-one induced DNA repair activity in primary rat hepatocytes following incubation *in vitro* with the chemicals (Goldsworthy et al. 1991). Administration of a single oral dose of 1,4-dioxane to Fischer 344 rats produced no evidence of hepatocyte DNA repair, did not increase DNA synthesis, relative liver weight or the activity of palmitoyl CoA oxidase (an indicator of peroxisome proliferation) (Goldsworthy et al. 1991). Furthermore, administration of a single dose of 1,000 mg/kg of 1,4-dioxane did not increase the hepatocyte labeling index 24 or 48 hours after dosing, but exposure to 1% 1,4-dioxane in the drinking water for 2 weeks resulted in a 2-fold increase in hepatic labeling index (Goldsworthy et al. 1991); the latter suggested that cell proliferation may play a role in the induction of liver carcinoma. In addition, no DNA repair activity or evidence of cells proliferation was

observed in nasal epithelial cells from rats administered 1% 1,4-dioxane in the drinking water for 2 weeks (Goldsworthy et al. 1991). However, it must be mentioned that Goldsworthy et al. (1991) acknowledged that a 2-week period of exposure might have been too short for detecting a proliferative response. Goldsworthy et al. (1991) concluded that the mechanism of carcinogenicity of 1,4-dioxane remains obscure and may involve a novel mechanism.

Gold et al. (1993) analyzed 351 chemicals in the Carcinogenic Potency Database (CPDB) and pointed out that, relative to non-mutagenic chemicals, mutagens are more likely to be carcinogenic, more likely to induce tumors at multiple target sites and, more likely to be carcinogenic in two species. Since 1,4-dioxane was carcinogenic in more than one species and induced tumors at multiple sites, one would expect that 1,4-dioxane would behave like a mutagen, but the empirical data suggest otherwise. Gold (1993) pointed out that among the CPDB chemicals tested for mutagenicity 75% are mutagens compared to 45% for non-mutagens and suggested that administration of large doses in cancer bioassays result in mitogenesis, which in turn increases the rate of mutagenesis and thus carcinogenesis.

The lack of significant genotoxicity along with the cytotoxicity observed at dose levels that induce tumors support the view that 1,4-dioxane acts via an unknown non-genotoxic mechanism.

The mechanism(s) by which 1,4-dioxane induces kidneys lesions is not known and virtually no discussion about this topic was found in the available reviews. The findings in the cases described by Barber (1934) and Johnstone (1959) are consistent with an acute nephritic syndrome, which is characterized by acute renal failure and oliguria. The impaired glomerular filtration rate causes extracellular fluid volume expansion, edema, and hypertension. A study of controlled exposures in volunteers showed that 1,4-dioxane has poor renal clearance, 0.34 mL/minute (Young et al. 1977), which probably contributes to accumulation of the chemical in the kidneys as biotransformation to the metabolite HEAA becomes saturated under conditions of high exposure.

3.5.3 Animal-to-Human Extrapolations

Exposure to high concentrations of 1,4-dioxane induces liver and kidneys effects in humans and in animals, regardless of the route of exposure. Based solely on the similarity of target organs, it would appear that results from animal studies could be used as valid models to predict health effects in humans resulting from high-dose exposure to 1,4-dioxane.

Long-term oral exposure of rodents to 1,4-dioxane has induced liver tumors in rats and mice as well as tumors in the nasal cavity of rats (JBRC 1998c; Kociba et al. 1974; NCI 1978). Therefore, the issue is whether these long-term, relatively high-exposure studies in animals are relevant to the low environmental exposures to 1,4-dioxane experienced by the general population. Studies of humans exposed chronically to relatively low concentrations of 1,4-dioxane in the air in occupational settings have provided no evidence of ill effects among the workers, including cancer, associated with 1,4-dioxane (Buffler et al. 1978; Thiess et al. 1976). However, it is unclear if the effects reported in humans are consistent with the potency estimated in rodents.

Some studies have shown that the liver tumors in rats are accompanied by extensive toxicity, as evidenced by hepatocyte hyperplasia, accumulation of fat in the cytoplasm, and degenerative changes (JBRC 1998c; Kociba et al. 1974; NCI 1978), which has led some to suggest that cell damage and degeneration may be a necessary occurrence for the formation of liver tumors in rats. Since liver toxicity in rats seems to occur only at dose levels at which plasma clearance and excretion of HEAA are reduced, and plasma concentrations of unchanged 1,4-dioxane are increased, it may be appropriate to consider the differences in metabolic disposition when extrapolating from effects that occur only with high doses to low-dose events (Kociba et al. 1975).

The relevance to humans of the nasal lesions and nasal tumors consistently seen in rats following exposure to 1,4-dioxane through the drinking water in many studies has been questioned (Stickney et al. 2003). Goldsworthy et al. (1991) suggested that the tumors resulted from inspiration of water containing 1,4-dioxane into the nasal cavity. Preliminary studies with a dye in the drinking water demonstrated that large amounts of inhaled water may be deposited directly in the nose as the animals drink (Reitz et al. 1990). The lack of nasal tumors in mice in chronic drinking water studies could be due to differences in tissue sensitivity and/or repair mechanisms, or to differences in anatomical features. However, species differences are difficult to establish since 1,4-dioxane acts via an unknown mechanism to produce tumors in liver, nasal cavity, and other sites.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals

with this type of activity are most commonly referred to as endocrine disruptors. However, appropriate terminology to describe such effects remains controversial. The terminology endocrine disruptors, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning endocrine disruptors. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as hormonally active agents. The terminology endocrine modulators has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997b). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Based on the available information, there is no evidence that 1,4-dioxane is an endocrine disruptor in humans or in animals, but appropriate tests have not been conducted. The only relevant information that was located is that 1,4-dioxane tested negative for estrogenic activity in a reporter gene expression assay using yeast cells (Nishihara et al. 2000). A substance was considered positive when its activity was more than 10% of the activity of 10^{-7}M 17β-estradiol.

Long-term oral studies have found no histopathologic (non-neoplastic) alterations in endocrine glands and reproductive organs from rats and mice (Kociba et al. 1974; NCI 1978), and the same was found in a chronic-duration inhalation study in rats (Torkelson et al. 1974). However, neoplasms associated with the

administration of 1,4-dioxane occurred in the testis/epididymis in male rats administered ≥240 mg 1,4-dioxane/kg/day in the drinking water for 2 years (NCI 1978). Another 2-year bioassay reported an increased incidence of mammary gland adenomas in rats treated in the drinking water with 514 mg 1,4-dioxane/kg/day (JBRC 1998c).

Standard reproductive toxicity studies on 1,4-dioxane were not located and only one study that examined the developmental effects of 1,4-dioxane was available (Giavini et al. 1985). The latter reported slight fetotoxicity occurring at a dose level that also affected the mothers.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and in vitro models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are

proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

There are no studies that specifically address the health effects of exposure to 1,4-dioxane in children or in immature animals; therefore, it is unknown whether children differ from adults in their susceptibility to health effects from 1,4-dioxane. Data in adults were derived from occupational studies (Barber 1934; Buffler et al. 1978; Thiess et al. 1976) and studies in volunteers (Fairley et al. 1934; Silverman et al. 1946; Yant et al. 1930; Young et al. 1977). The former showed that exposure to high concentrations of 1,4-dioxane in the air (and also dermally) can severely damage the liver and kidneys and can be lethal. The studies of controlled exposure with volunteers showed that exposure to 1,4-dioxane in the air can produce eye, nose, and throat irritation. It is reasonable to assume that the same types of effects would be seen in children accidentally exposed to high amounts of 1,4-dioxane.

There is no information regarding possible adverse developmental effects in humans exposed to 1,4-dioxane. A study in rats exposed orally to 1,4-dioxane during gestation found slight fetotoxicity, but at a dose level that also affected the mothers (Giavini et al. 1985). There is evidence that 1,4-dioxane is at

most a weak genotoxic compound. Therefore, it is unlikely that parental exposure would result in adverse childhood development or cancer development as a result of 1,4-dioxane metabolite exposures to parental germ cells.

There is no information regarding pharmacokinetics of 1,4-dioxane in children. Analysis of urine samples from humans exposed to 1,4-dioxane suggests the involvement mainly of phase I metabolic enzymes in the biotransformation and elimination of 1,4-dioxane. The specific P-450 isozymes involved in phase I metabolism are not known, and thus, no conclusions can be drawn based on general differences in isozymes activities between adults and children. However, if the enzymes in question are lower in neonates and oxidation of 1,4-dioxane is a detoxification reaction, this could result in greater toxicity in neonates. It is not known whether 1,4-dioxane can cross the placenta and there are no reports on levels of 1,4-dioxane in maternal milk.

There are no biomarkers of exposure or effect for 1,4-dioxane that have been validated in children or in adults exposed as children. No relevant studies were located regarding interactions of 1,4-dioxane with other chemicals in children or adults.

No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to 1,4-dioxane, reducing body burden, or interfering with the mechanisms of action for toxic effects.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and

interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 1,4-dioxane are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by 1,4-dioxane are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations that are Unusually Susceptible.”

3.8.1 Biomarkers Used to Identify or Quantify Exposure to 1,4-Dioxane

1,4-Dioxane and its metabolite, HEAA, were found in the urine of workers exposed to a time-weighted average air concentration of 1.6 ppm of 1,4-dioxane for 7.5 hours (Young et al. 1976). The concentration of HEAA was 414 µmol/L and that of unchanged 1,4-dioxane was only 3.5 µmol/L, suggesting rapid and extensive metabolism. 1,4-Dioxane in the urine is a specific biomarker for exposure to 1,4-dioxane, but HEAA can also be produced by exposure to 1,4-dioxane-2-one and diethylene glycol. In a controlled-exposure study with volunteers exposed to 50 ppm 1,4-dioxane vapors for 6 hours, the half-life for elimination of 1,4-dioxane from plasma was 59 minutes (Young et al. 1977). The plasma concentration

of HEAA reached a peak at about 1 hour after exposure ceased and decreased linearly thereafter. Of all the 1,4-dioxane detected in the urine within a 48-hour period, 90% was excreted during the exposure period and none could be detected 6 hours after termination of the exposure. The half-life for elimination of 1,4-dioxane in the urine was 48 minutes, and that of HEAA was 2.7 hours. Almost all the 1,4-dioxane was excreted in the urine as HEAA. About half of the total HEAA excreted was excreted during the exposure period and the excretion was complete 18 hours after the exposure ceased. A simulation of repeated exposures to 50 ppm 1,4-dioxane for 8 hours/day showed that 1,4-dioxane will reach a peak in plasma at the end of each exposure day and will not accumulate; neither will HEAA. Collectively, these results imply that 1,4-dioxane and HEAA in plasma and urine can be used as biomarkers of recent isolated exposure or multiple daily exposures, but that could not differentiate between the two types of exposure (providing the exposure concentrations are below about 50 ppm). In addition, because these substances are rapidly eliminated, they cannot be used as biomarkers of past exposure to 1,4-dioxane. Given the low levels of 1,4-dioxane reported in the environment, it is not unlikely that the levels of 1,4-dioxane and HEAA in members from the general population fall under the detection levels of the available analytical methods.

Some chemicals bind to macromolecules (i.e., DNA, hemoglobin, etc.) to form compounds that can be used as specific biomarkers of exposure. That is not the case for 1,4-dioxane. In liver preparations from rats administered a single intraperitoneal dose of radioactive 1,4-dioxane, most of the radioactivity was bound non-covalently in the cytosol (Woo et al. 1977b). Covalent binding to macromolecules was highest in nuclear fraction followed by mitochondrial, microsomal, whole homogenate, and cytosol fractions. The binding was nonspecific and not associated with DNA. Pretreatment of rats with microsomal enzyme inducers had no significant effect on the covalent binding to macromolecules. There was no microsomally-mediated binding of radioactivity to DNA.

3.8.2 Biomarkers Used to Characterize Effects Caused by 1,4-Dioxane

The liver and kidneys are targets for 1,4-dioxane toxicity, but lesions to these organs cannot be considered specific biomarkers for 1,4-dioxane because exposure to many different chemicals or health conditions unrelated to chemical exposures can produce similar effects.

3.9 INTERACTIONS WITH OTHER CHEMICALS

The only information located that is relevant to environmental or occupational exposures is that from a study by Buffler et al. (1978) in workers, even though it provides only suggestive evidence that interactions may have played a role in the outcome. In a cohort of 165 workers exposed intermittently to concentrations of 1,4-dioxane between 0.1 and 17 ppm, seven deaths were identified among those working in the manufacturing area and five among those involved in the processing area (Buffler et al. 1978). The exposure histories of the seven subjects indicated that all were exposed to other chemicals of possible significance at earlier times and for longer intervals than their exposure to 1,4-dioxane. In addition, the five deaths that occurred among the processing area were exposed to vinyl chloride simultaneously with their exposure to 1,4-dioxane. No firm conclusions can be drawn from this study regarding interactions of 1,4-dioxane with other chemicals.

If cytochrome CYP2E1 is involved in the metabolism of 1,4-dioxane (cytochrome P-450 is known to be involved in 1,4-dioxane metabolism, but the specific isozymes are not known), then ethanol could alter the hepatic effects of 1,4-dioxane if one assumes that the toxic entity is a metabolite of 1,4-dioxane. In the Thiess et al. (1976) study, some workers exposed to 1,4-dioxane who consumed alcohol frequently had elevated serum levels of transaminases; however, the values became normal after the workers reduced their alcohol consumption, suggesting that the elevated transaminase values were purely due to exposure to ethanol and not to the combination of 1,4-dioxane and ethanol, at least at the relative low level of exposure experienced by the workers in this occupational study (maximum 14.3 ppm).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 1,4-dioxane than will most persons exposed to the same level of 1,4-dioxane in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of 1,4-dioxane, or compromised function of organs affected by 1,4-dioxane. Populations who are at greater risk due to their unusually high exposure to 1,4-dioxane are discussed in Section 6.7, Populations with Potentially High Exposures.

Because 1,4-dioxane is a liver and kidney toxicant at high concentrations, people with compromised liver or kidneys function may be more susceptible to the effects of exposure to 1,4-dioxane than healthy

individuals. Among those unusually susceptible would be, for example, individuals who drink excessive amounts of alcohol, those on medications known to affect the liver or the kidneys, or those with genetic diseases of the kidney.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 1,4-dioxane. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to 1,4-dioxane. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

No texts were found that provided specific information about treatment following exposure to 1,4-dioxane.

3.11.1 Reducing Peak Absorption Following Exposure

The only relevant information that was located is that the skin and eyes should be immediately flushed with water for at least 15 minutes following skin and eye contact (NIOSH 1977). If 1,4-dioxane is swallowed, vomiting should be induced immediately if the patient is conscious (NIOSH 1977).

3.11.2 Reducing Body Burden

No information was located regarding reducing body burden following exposure to 1,4-dioxane. As mentioned in Section 3.4, Toxicokinetics, 1,4-dioxane and its main metabolites do not accumulate and are rapidly eliminated from the body in the urine.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The liver and kidneys are targets for 1,4-dioxane toxicity in humans and animals. Lesions have been found in humans acutely exposed to relatively high concentrations of 1,4-dioxane and in animals following inhalation, oral, and dermal exposure (see Section 3.2). Also, 1,4-dioxane has induced liver

cancer in rats and mice and nasal cancer in rats. The mechanism(s) of toxic action of 1,4-dioxane has not been elucidated, but there is increasing evidence that the liver lesions seen in animals evolve into neoplasms induced by 1,4-dioxane through a non-genotoxic mechanism of action. Any attempt to discuss possible mechanisms to interfere with this action would be pure speculation at this time.

3.12 ADEQUACY OF THE DATABASE

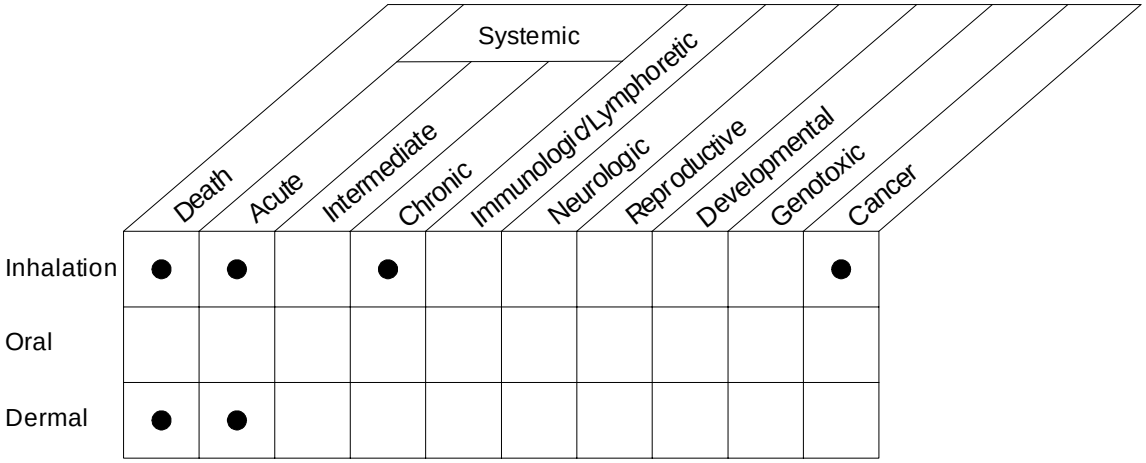
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 1,4-dioxane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 1,4-dioxane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

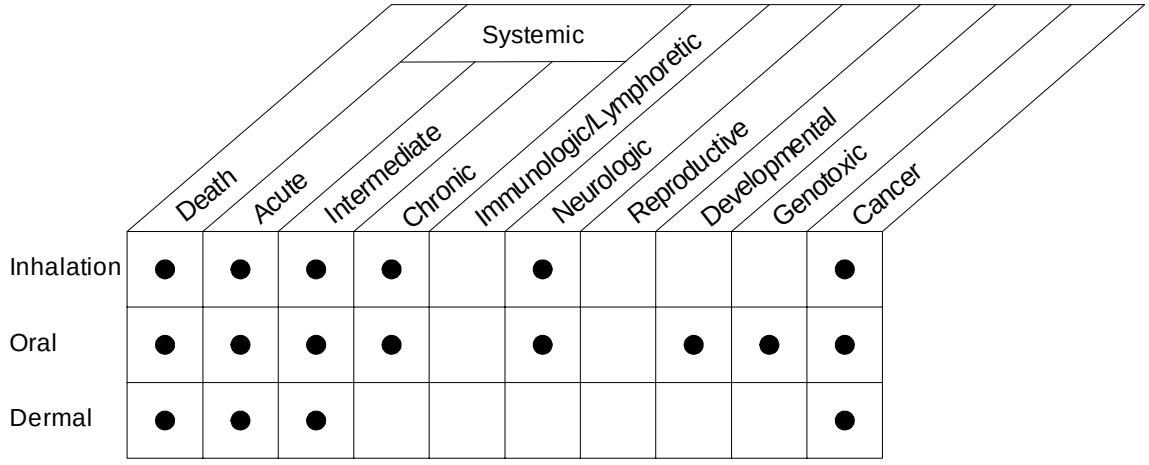
3.12.1 Existing Information on Health Effects of 1,4-Dioxane

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 1,4-dioxane are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of 1,4-dioxane. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a “data need”. A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 3-5. Existing Information on Health Effects of 1,4-Dioxane



Human



Animal

● Existing Studies

As shown in Figure 3-5, there is limited information on the effects of 1,4-dioxane in humans. The available information is derived from occupational studies in which exposure was assumed to have been primarily by inhalation of vapors, but that may have also involved dermal exposure. These studies provided information on acute systemic effects and lethality and also effects due to long-term exposure. A few studies of controlled inhalation exposures with volunteers are also available and these studies provided data on acute systemic effects. No information was located regarding oral exposure of humans to 1,4-dioxane.

In animals, the studies available for review provided information on lethality and on systemic, neurological, and cancer effects following inhalation exposure to 1,4-dioxane. For oral exposure, there are studies that evaluated systemic, neurological, developmental, genotoxic, and cancer effects. No studies were available regarding chronic systemic effects, or immunological, neurological, reproductive, developmental, or genotoxic effects after dermal exposure to 1,4-dioxane.

The information available from human and animals studies suggests that the effects of 1,4-dioxane are not route-dependent. In addition, the limited environmental monitoring data available suggests that the levels of 1,4-dioxane to which the general population might be exposed through contact or use of consumer products (including food), or that are normally found in environmental media are generally orders of magnitude lower than those used in studies with experimental animals.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Two occupational studies provided acute inhalation data for 1,4-dioxane, Barber (1934) and Johnstone (1959). Barber (1934) described five lethal cases among factory workers exposed to 1,4-dioxane, whereas Johnstone (1959) described one additional lethal occupational case in which dermal exposure also occurred. Exposure to unknown, but lethal concentrations of 1,4-dioxane produced serious liver and kidney effects. A few additional studies in volunteers evaluated mostly clinical signs, such as eye and nose irritation, during exposures varying from 3 minutes to 6 hours (Fairley et al. 1934; Silverman et al. 1946; Yant et al. 1930; Young et al. 1977). The lowest concentration that produced an effect was 50 ppm during a 6-hour exposure, which caused eye irritation (Young et al. 1977). Data from the study by Young et al. (1977) were used to derive an acute-duration inhalation MRL for 1,4-dioxane. The animal database consists mainly of early studies in rodents exposed to lethal or near lethal concentration of 1,4-dioxane that indicated that the liver and kidneys are

the main targets of 1,4-dioxane toxicity in animals (Fairley et al. 1934; Yant et al. 1930). Additional acute inhalation studies conducted according to current guidelines would be helpful to establish dose-response relationships for liver and kidney effects at low levels of exposure. No data were located regarding acute oral exposure of humans to 1,4-dioxane. Most studies in animals provided lethal dose levels and also showed that the liver and kidneys are the organs most severely affected by high oral doses of 1,4-dioxane (de Navasquez 1935; Kesten et al. 1939; Laug et al. 1939; Schrenk and Yant 1936; Smyth et al. 1941). A more recent 2-week drinking water study in rats, although with limitations, provided sufficient information on systemic end points and was used as the basis for derivation of an acute-duration oral MRL for 1,4-dioxane (JBRC 1998a). Additional acute oral studies conducted according to current guidelines could provide information on thresholds for liver and kidney effects. Also, exposures to low or moderate single oral doses followed by long observation periods would provide information on reversibility of the effects. Limited acute dermal data were found. In the lethal occupational case described by Johnstone (1959), considerable dermal exposure occurred since the subject used to wipe his hands with 1,4-dioxane to clean them; this probably contributed to the liver and kidney toxicity observed. In the studies with volunteers mentioned above, eye irritation was most likely due to direct contact of the eye with the vapors of 1,4-dioxane and not due to inhaled 1,4-dioxane. A study in rats applied a dose of 8,300 mg/kg of 1,4-dioxane to a shaved area of the skin found no signs of skin irritation during a 14-day observation period (Clark et al. 1984). Additional acute dermal studies may be tied to studies of the pharmacokinetics of 1,4-dioxane by this route of exposure, which has not been well characterized.

Intermediate-Duration Exposure. No intermediate-duration studies in humans were available. An early study by Fairley et al. (1934) in several animal species provided enough information to determine that the liver and kidneys are targets for 1,4-dioxane toxicity. The lowest concentration of 1,4-dioxane to which rats, mice, and guinea pigs were exposed intermittently for 3–12 weeks was 1,000 ppm, which caused moderate to severe kidney toxicity. Because of the severity of the effects, this information was considered inadequate for derivation of an intermediate-duration inhalation MRL. However, the chronic-duration inhalation MRL (see below) was also adopted as the intermediate-duration inhalation MRL. Several oral studies in animals provided information on lethal doses (Fairley et al. 1934; Kociba et al.1974) and on systemic effects, mostly hepatic and renal (Fairley et al. 1934; Lundberg et al. 1987; Stott et al. 1981). A more recent 90-day drinking water study in rats provided sufficient information on multiple end points and was used as the basis (liver effects) for an intermediate-duration oral MRL for 1,4-dioxane (JBRC 1998b). Information by the dermal route of exposure was limited to a study of intermittent application of 1,4-dioxane to the skin of rabbits and guinea pigs for up to 101 days (Fairley et al. 1934). There were no dermal effects in either species at dose levels that induced liver and kidney

lesions, which appeared to be more severe in rabbits than in guinea pigs. Although the target organs following inhalation exposure are known, additional inhalation studies with lower concentrations of 1,4-dioxane and following current testing standards are needed to establish dose-response relationships for liver and kidney effects and to obtain more information on possible effects on other organs (e.g., reproductive organs, endocrine glands). Information on effects on other organs is also limited in intermediate oral studies. The use of PBPK models for 1,4-dioxane (Leung and Paustenbach 1990; Reitz et al. 1990) may obviate the need for additional studies by multiple routes of exposure, since target organ concentrations obtained following exposure by one route may be used to back estimate exposure concentrations by a different route.

Chronic-Duration Exposure and Cancer. An occupational study of workers exposed to 1,4-dioxane provides information regarding long-term exposure to this chemical. Thiess et al. (1976) found no adverse effects in workers exposed to 0.006–14.3 ppm 1,4-dioxane for an average of 25 years. Only one chronic-duration study by the inhalation route was available (Torkelson et al. 1974). This study provided information on multiple organs and tissues, and hematology parameters in rats; no adverse effects were found. This study was used to derive a chronic-duration inhalation MRL for 1,4-dioxane. Because only one exposure level was used, the true NOAELs for the various organs and systems are unknown and may be higher. Therefore, additional studies in animals exposed to higher concentrations of 1,4-dioxane may be necessary to fill this data gap. Also, as mentioned above, PBPK models may be used to extrapolate data from the chronic oral studies to inhalation exposure situations. Several chronic-duration oral studies in rats and mice are available (JBRC 1998c; Kociba et al. 1974; NCI 1978). These studies provided information on clinical signs, changes in body weight, hematology, blood chemistry, urinalysis, and gross and microscopic appearance of major organs and tissues. The liver and kidneys were the main targets for 1,4-dioxane toxicity. A NOAEL of 9.6 mg/kg/day for liver effects in male Sherman rats was used to derive a chronic-duration oral MRL for 1,4-dioxane (Kociba et al. 1974). Additional chronic oral studies do not seem necessary at this point. No chronic dermal studies were located, but it is not apparent what new key information such studies could provide.

Very limited information was found regarding human exposure to 1,4-dioxane and cancer. A study of 165 workers exposed intermittently to 0.1–17 ppm 1,4-dioxane for up to 21 years found no significant increases in the incidences of deaths due to cancer (Buffler et al. 1978). 1,4-Dioxane was not carcinogenic in rats in the only single inhalation bioassay (Torkelson et al. 1974). However, only one exposure level was used; therefore, a dose-response relationship for cancer could not be estimated. Long-term oral administration of 1,4-dioxane induced liver cancer in rats and mice and also nasal tumors in rats

(Argus et al. 1965, 1973; Hoch-Ligeti et al. 1970; JBRC 1998c; Kociba et al. 1974; NCI 1978). 1,4-Dioxane was not a complete carcinogen in a 60-week dermal exposure study in mice (King et al. 1973), but showed promoter activity in oral (Lundberg et al. 1987) and dermal studies (King et al. 1973). 1,4-Dioxane was not an initiator in a dermal assay in mice (Bull et al. 1986). Additional bioassays will probably not provide any new key information at this time, but since the mechanism of carcinogenicity of 1,4-dioxane is yet unknown, continued research on this topic and on the role of metabolism in carcinogenicity is necessary. Some have suggested that the cancer risk assessment for 1,4-dioxane be updated (Stickney et al. 2003). Specifically, it has been suggested that the nasal tumors in rats, which is the basis for the current oral slope factor derived by EPA (IRIS 2004), are not relevant for human exposure because they result from water entering the nasal cavity when the animals drink from sipper bottles. Also, the PBPK modeling studies of Leung and Paustenbach (1990) and Reitz et al. (1990) have been applied to estimate the internal dose and the potential human cancer risks. Finally, the use of a nonlinear approach to low dose extrapolation might be considered based on the observations that liver toxicity, which some have suggested may be required for tumor development, occurs only at doses above which the metabolism of 1,4-dioxane is saturated. The EPA is currently re-evaluating the health assessment for 1,4-dioxane (EPA 2004f).

Genotoxicity. The genotoxic effects of 1,4-dioxane have been well characterized in studies *in vitro* in microorganisms (Haworth et al. 1983; Hellmer and Bolcsfoldi 1992; Khudoley et al. 1987; Kwan et al. 1990; Morita and Hayashi 1998; Nestmann et al. 1984; Stott et al. 1981; Zimmermann et al. 1985) and in mammalian cells (Galloway et al. 1987; Goldsworthy et al. 1991; McGregor et al. 1991; Morita and Hayashi 1998; Sheu et al. 1988). Most of these studies were conducted both in the presence and absence of metabolic activation systems, which would suggest that metabolites of 1,4-dioxane also are not mutagenic. The results from studies *in vivo* also provided mostly negative evidence of genotoxicity (Goldsworthy et al. 1991; Kitchin and Brown 1990, 1994; McFee et al. 1994; Mirkova 1994; Morita and Hayashi 1998; Muñoz and Barnett 2002; Stott et al. 1981; Tinwell and Ashby 1994; Yoon et al. 1985). The total weight of evidence suggests that 1,4-dioxane is either a weak genotoxin or not genotoxic, and it is unlikely that further studies will provide new information.

Reproductive Toxicity. No information was located regarding reproductive effects of 1,4-dioxane in humans. There are studies that examined the gross and microscopic appearance of the reproductive organs from rats following chronic inhalation exposure (Torkelson et al. 1974) and from rats and mice following intermediate oral exposure (JBRC 1998b) and chronic oral exposure to 1,4-dioxane (JBRC 1998c; Kociba et al. 1974; NCI 1978), but no assessments of reproductive function or examinations of

sperm characteristics have been made. The lack of effects on reproductive organs observed in these studies diminishes the need to conduct a 2-generation reproductive study. In addition, only one study was located that tested the estrogenic properties of 1,4-dioxane in an assay *in vitro* (Nishihara et al. 2000), with negative results. Additional standard *in vivo* and *in vitro* studies to assess whether 1,4-dioxane has endocrine disruptor properties would be useful.

Developmental Toxicity. There is no information on developmental effects in humans exposed to 1,4-dioxane. If populations were identified that are exposed to high levels of 1,4-dioxane, it would be useful to determine whether 1,4-dioxane or metabolites are found in breast milk. This can also be done in surveys monitoring chemicals in the general population at the national level. Only one study was located that evaluated developmental parameters in rats exposed orally by gavage during gestation (Giavini et al. 1985). Slight fetotoxicity was seen at a dose level that affected the mothers. Additional studies are necessary to determine whether adverse developmental effects can occur without maternal toxicity. In addition, a developmental neurotoxicity study in rats in which pups are tested at various ages after being exposed *in utero* and/or via maternal milk would fill a data gap.

Immunotoxicity. No information was located regarding immunotoxic effects in humans following exposure to 1,4-dioxane. The information from animal studies is restricted to gross and microscopic examination of lymph nodes and the spleen from rats exposed intermittently to 111 ppm 1,4-dioxane vapors for 2 years (Torkelson et al. 1974) and of the lymph nodes, spleen, and thymus from rats and mice dosed with up to 2,700 mg 1,4-dioxane/kg/day in the drinking water for 13 weeks (JBRC 1998b), or in rats and mice dosed with up to 1,599 mg 1,4-dioxane/k/day in the drinking water for up to 2 years (JBRC 1998c; Kociba et al. 1974; NCI 1978). No treatment-related effects were observed. Although there was no indication that immunocompetence was compromised in these studies, a study performing a complete Tier I battery of tests may be warranted to evaluate the possibility that exposure to 1,4-dioxane might cause subtle alterations in immune parameters.

Neurotoxicity. Edema of the brain was observed in lethal cases of intoxication with 1,4-dioxane vapors (Barber 1934; Johnstone 1959). Occupational studies of long-term exposure to lower concentrations of 1,4-dioxane did not report signs or symptoms that would indicate neurological damage, but sensitive tests were not conducted (Buffler et al. 1978; Thiess et al. 1976). Exposure of mice and rats for 4 hours to 1,800–2,400 ppm 1,4-dioxane had a narcotic effect (Frantik et al. 1994) and exposure to 3,000 ppm intermittently for 2 weeks affected an avoidance response in rats (Goldberg et al. 1964), which also could have been due to narcosis. High oral doses also induced narcosis in rabbits (Knoefel 1935).

Vacuolar changes were observed in the brain from rats exposed to 2,750–2,960 mg 1,4-dioxane/kg/day for 2 weeks (JBRC 1998a) and from rats exposed to 1,900–2,010 mg/kg/day for 13 weeks (JBRC 1998b). Long-term inhalation (Torkelson et al. 1974) and oral studies (JBRC 1998c; Kociba et al. 1974; NCI 1978) in rats and mice have provided no indication of adverse clinical signs in the animals and examination of the brain, spinal cord, and sciatic nerve was unremarkable. The overall information suggests that 1,4-dioxane may have narcotic properties at high concentrations, but it would be useful to determine whether possible subtle behavioral effects can be detected with more sensitive tests at exposure concentrations that do not induce narcosis.

Epidemiological and Human Dosimetry Studies. Information on the health effects of 1,4-dioxane in humans is derived from cases of accidental exposure at work to relatively high concentrations of 1,4-dioxane, which caused death (Barber 1934; Johnstone 1959), and studies of long-term exposure, also at work, to lower concentrations of 1,4-dioxane (Buffler et al. 1978; Thiess et al. 1976). Follow-up evaluations of individuals who may have been occupationally exposed would provide valuable information. No specific group from the general population that may have been subjected to unusually high amounts of 1,4-dioxane was identified. If such a situation arises, for example due to an accidental spill or leak from a waste site resulting in contaminated water or soil, individuals potentially exposed to 1,4-dioxane should be monitored for liver and kidney effects with standard function tests, since the liver and the kidneys have been identified as targets for 1,4-dioxane toxicity.

Biomarkers of Exposure and Effect.

Exposure. 1,4-Dioxane and its main metabolite, HEAA, have been identified in the blood and urine from workers exposed to 1,4-dioxane vapors (Young et al. 1976) and from volunteers exposed to controlled amounts 1,4-dioxane vapors (Young et al. 1977). Under condition of low to moderate exposure, the transformation of 1,4-dioxane to HEAA is rapid and extensive, and HEAA is rapidly eliminated in the urine (Young et al. 1977). The development of models that would support quantitative estimates of exposure to 1,4-dioxane based on urine levels of HEAA may be valuable in cases of high exposure, but given the very low levels of 1,4-dioxane that the general population is exposed to, the development of analytical methods capable to detect and quantify HEAA in the general population may be more useful.

Effect. There are no biomarkers of effect specific for 1,4-dioxane. Exposure to high amounts of 1,4-dioxane affects the liver and kidneys, but no 1,4-dioxane-induced health effects have been reported in

populations exposed to low amounts of 1,4-dioxane (Buffler et al. 1978; Thiess et al. 1976). Research to identify reliable biomarkers for exposure to 1,4-dioxane in humans would be useful in order to evaluate the prevalence and magnitude of exposure in an at-risk population.

Absorption, Distribution, Metabolism, and Excretion. Among the areas of absorption, distribution, metabolism, and excretion, the greatest data need lies in metabolism; specifically, the determination of the metabolic pathways involved in the metabolism of 1,4-dioxane to its primary metabolite, HEAA or 1,4-dioxane-one (Braun and Young 1977; Woo et al. 1977a, 1977b, 1977c; Young et al. 1977). While the identity of the metabolite has been determined and the involvement of cytochrome P-450 enzymes has been demonstrated (Woo et al. 1977c, 1978), the formation of intermediate metabolites, and their identities, has not been demonstrated. Additional information regarding this pathway may be useful in the refinement of PBPK models and in the development of biomarkers of exposure and/or effect. Data are lacking on the absorption of 1,4-dioxane in humans following oral and dermal exposure, but this information would likely do little to further our understanding of the pharmacokinetic processes of 1,4-dioxane.

Comparative Toxicokinetics. Studies directly comparing the toxicokinetics of 1,4 dioxane across species are not available. Some limited data on 1,4-dioxane absorption following inhalation exposure suggest large differences in the absorbed dose, expressed on a per body weight basis, between rats and humans (Young et al. 1977, 1978a, 1978b). However, these studies did not measure absorption efficiencies. Studies examining absorption efficiency in humans and rats following inhalation and oral exposures would provide valuable data for evaluating possible species differences. The available data on metabolism and elimination of 1,4 dioxane in humans and rats indicate that the compound behaves similarly in the two species (Woo et al. 1977a, 1977b, 1977c, 1978; Young et al. 1976, 1977, 1978a, 1978b). The available PBPK models for 1,4 dioxane also indicate that the behavior of 1,4 dioxane is similar in rats and humans. Studies of the toxicokinetic behavior of 1,4 dioxane in animal species other than the rat would provide additional insight into potential interspecies differences, while studies directly comparing the toxicokinetic behavior of 1,4 dioxane in multiple species would add to our understanding of the comparative toxicokinetic behavior of 1,4 dioxane.

Methods for Reducing Toxic Effects. No specific methods for the mitigation of effects of acute exposure to 1,4-dioxane were located other than measures to support vital functions. No information was located concerning mitigation of effects of lower-level or longer-term exposure to 1,4-dioxane. This, in part, may reflect the fact that no population has been identified as having been subjected or currently

undergoing exposure to excessive amounts of 1,4-dioxane. Attempts to propose studies of specific methods to reduce possible adverse effects do not appear warranted at this time.

Children's Susceptibility. There are no studies that specifically addressed exposure to 1,4-dioxane in children. Workers exposed to high amounts of 1,4-dioxane vapors experienced liver and kidney effects and some died (Barber 1934; Johnstone 1959). Volunteers exposed to low concentrations of 1,4-dioxane in the air experienced eye and nose irritation (Silverman et al. 1946; Yant et al. 1930; Young et al. 1977). It is reasonable to assume that children exposed in similar manners will experience similar effects. There is no information on whether the developmental process is altered in humans exposed to 1,4-dioxane. Very limited evidence with 1,4-dioxane in rats suggests that fetotoxicity may occur only at maternally toxic levels (Giavini et al. 1985), but further studies are necessary on this issue. The possibility that 1,4-dioxane may have endocrine-disrupting ability in mammals has not been systematically studied.

There are no data to evaluate whether pharmacokinetics of 1,4-dioxane in children are different from adults. There is no information on whether 1,4-dioxane can cross the placenta and there are no studies on whether 1,4-dioxane can be transferred from mother to offspring through maternal milk. Cross-fostering studies can provide important information regarding the role of *in utero* vs. lactation exposure to 1,4-dioxane in normal development. There are no data to permit an evaluation of whether metabolism of 1,4-dioxane is different in children than in adults.

Research into the development of sensitive and specific biomarkers of exposures and effects for 1,4-dioxane would be valuable for both adults and children. There are no data on the interactions of 1,4-dioxane with other chemicals in children. There are no pediatric-specific methods to reduce peak absorption 1,4-dioxane, to reduce body burdens, or to interfere with the mechanisms of action. Based on the information available, it is reasonable to assume that the supportive methods recommended for maintaining vital functions in adults, will also be applicable to children.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

No ongoing studies pertaining to 1,4-dioxane were identified in the FEDRIP (2004) database.